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Technology, Public Policy, and the Changing Structure of American Agriculture

Volume II—Background Papers

Part A: The Emerging Technologies (First Section)

1. Genetic Engineering in Animals
2. Animal Reproduction
3. Regulation of Growth and Development
4. Animal Disease Control
5. Animal Nutrition
6. Livestock Pest Control
7. Environment and Animal Behavior
8. Crop Residue and Animal Waste Use
9. Genetic Engineering in Plants
10. Enhancement of Photosynthetic Efficiency
11. Plant Growth Regulators
12. Plant Disease and Nematode Control
13. Management of Insects and Mites
14. Biological Nitrogen Fixation
15. Water and Soil-Water-Plant Relations

May 1986

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Volume II--Background Papers

Part A: The Emerging Technologies (First Section)

Paper No.

1. Genetic Engineering in Animals by Howard L. Bachrach
2. Animal Reproduction by William Hansel
3. Regulation of Growth and Development by Eugene C. Allen
4. Animal Disease Control by Bennie I. Osburn
5. Animal Nutrition by Wilson G. Pond
6. Livestock Pest Control by J.B. Campbell
7. Environment and Animal Behavior by Stanley E. Curtis
8. Crop Residue and Animal Waste Use by James R. Fischer
9. Genetic Engineering in Plants by Robert T. Fraley
10. Enhancement of Photosynthetic Efficiency by Joseph A. Berry
11. Plant Growth Regulators by Louis G. Nickell
12. Plant Disease and Nematode Control by J.A. Browning
13. Management of Insects and Mites by George G. Kennedy
14. Biological Nitrogen Fixation by Martin Alexander
15. Water and Soil-Water-Plant Relations by Larry Boersma and E.L. McCoy

Background Paper No. 1

Genetic Engineering in Animals

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Office of Technology Assessment
U.S. Congress

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Summary

Genetic engineering consists of a number of procedures by which genes can be manipulated for improving the health and productivity of plants, animals, and humans. Three important genetically engineering procedures are: 1) recombinant DNA (rDNA), also called gene splicing, 2) monoclonal antibody production, and 3) embryo transfer. Because of its power to alter life forms, rDNA technology is considered one of the greatest achievements of biological science. Any isolated DNA gene can be spliced into a carrier DNA molecule producing a rDNA molecule for insertion into a bacterium, yeast, or animal cell, where the guest gene is made to replicate and produce its protein product. Many useful proteins are being produced in this manner: insulin, growth hormones, prolactin, prolaxin, enzymes, toxins, blood proteins, subunit protein vaccines, immunity enhancers such as interferons and interleukins, and nutrients like amino acids and single-cell protein feed supplements. Also, rDNA technology produces DNA sequences for use as probes in detecting bacterial poisoning of foods and for diagnosing and treating of infectious and genetic diseases.

A monoclonal antibody is unique in that it reacts with a single specific site on the foreign antigen used to immunize the animal. Monoclonal antibodies are made by fusing antibody-producing cells from the animal with myeloma cells: the fused cells called hybridomas produce (theoretically in perpetuity) large quantities of monoclonal antibodies. The many important uses of monoclonal antibodies include: the purification of proteins made by rDNA; passive immunization of calves against scours; detection of food poisoning; substitutes for vaccines, antitoxins and antivenoms; sexing of livestock embryos; post-coital contraception and pregnancy testing; imaging,

targeting and killing of cancer cells; monitoring levels of hormones and drugs; and prevention of rejection of organ transplants.

Embryo transfer is being used for the rapid upgrading of the quality and productivity of livestock, particularly of cattle. A superovulated donor animal is artificially inseminated, and the resulting embryos are removed nonsurgically for implantation in and carrying to term by surrogate mothers. Before implantation, the embryos can be treated in various ways. They can be sexed with monoclonal antibody, split to make twins, fused with embryos of other animal species or frozen in liquid nitrogen for storage until estrus in the surrogate mothers on farms is in synchrony with that of the donor. In addition, single-cell livestock embryos have been injected with cloned genes made by rDNA technology in an attempt to endow the germplasm with new or enhanced traits. This work stemmed from the observation that single-cell mouse embryos injected with cloned human growth hormone genes produce twice normal size mice that pass this trait onto succeeding generations. Gene insertions also have potential for treating genetic diseases.

Monoclonal antibody and rDNA techniques are pinpointing the molecular defects in genes that cause cancer and leukemias. Inacitve cancer genes have been found to be normally present in birds, animals and humans, and to be activated to produce cancer by a variety of chemicals, viruses, and irradiations. These advances should eventually help to control cancer in livestock and poultry, such as bovine and avian leukosis.

Because of intense competition among hundreds of firms in the U.S. and abroad, a great many useful genetically engineered products and processes will be introduced during the 1980s.

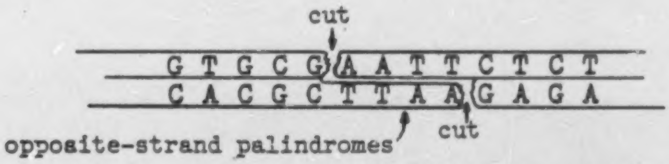
Introduction

Definition and Scope

Genetic engineering is comprised of a number of procedures by which genes can be manipulated for the purpose of improving the health and productivity of plants, animals, and humans. This paper describes the application of these procedures to animals. In addition, many applications of genetic engineering in humans will also be described, owing to the likelihood of comparable future uses in animals. Genetic engineering developed in the early 1970s principally from nearly simultaneous breakthroughs in three interrelated areas of biology: molecular genetics, immunology, and embryology. Originating in California, the breakthrough in molecular genetics permitted functional genes to be transplanted between plants, animals, and microorganisms -- events that occur rarely in nature, except for plasmid exchanges in microorganisms. Gene transplantation has several additional names: recombinant DNA (rDNA), gene splicing, gene cloning, molecular cloning, cell transformation, and genetic reprogramming. The breakthroughs in immunology (monoclonal antibody production in hybridomas) and in embryology (embryo transfer) were made in England.

Because of its power to alter life forms, rDNA technology must be ranked as one of the greatest achievements of biological science. A short description of the molecular processes involved in rDNA follows. It is fundamental that information for the reproduction, development and growth of animals is contained, as molecular recipes, in long double-stranded thread-like molecules of deoxyribonucleic acid (having the acronym DNA) situated in the nucleus of animal cells (see figure 1). The DNA threads have regions called genes, each of which is a recipe for making a specific protein molecule. (It is estimated that large animals and humans have about 100,000 different genes, i.e., recipes, in their cells.) However, as shown in figure 1, protein molecules are not made directly from DNA genes; rather, the recipes in genes are first copied individually by a process called transcription into short single-stranded thread-like ribonucleic acid molecules called messenger RNAs (mRNAs), much like xeroxing recipes out of a large cookbook. Owing partly to their small size, unlike the bulky DNA threads, mRNAs can migrate through the pores of the nuclear membrane (figure 1) into the cytoplasm (i.e., the cell substance between the nucleus and the cell's outer membrane) where their individual messages are converted on numerous bio-machines called ribosomes into specific protein molecules by a very intricate process called translation. The intracellular transcriptional and translational processes are analogous to reading and writing in human languages. However, DNA has only the 4-letter alphabet A, T, C, and G (representing the molecules adenine, thymine, cytosine, and quanine, respectively) forming the words of the recipes in the genes along the DNA threads; RNA is just a dialect of DNA, having a U (for uracil) instead of the T in DNA. Owing to molecular constraints in the transcription process, the DNA letters A, T, C, and G are transcribed, with exceedingly high fidelity, into the RNA letters U, A, G, and C, respectively. The translation process is more complicated. The question is: how do the letters in the mRNAs form words that specify the correct sequence of incorporation of each of 20 different amino acids into a growing protein chain? The answer (known as the genetic code, figure 2) is that the 64 possible 3-letter combinations of the 4 RNA letters form all of its words (called codons), and that each codon directs the insertion of a specific amino acid into a growing protein chain or terminates it. Only the codon AUG can initiate protein chains, causing the insertion of the amino acid methionine.

However, any of the three codons -- UAA, UAG, and UUGA -- can terminate chains. The genetic code has many more redundancies; for example, there are six codons each for the insertion of the amino acids leucine, serine, and arginine. The fact that the code was found to be essentially the same for all life forms was largely responsible for the idea that it might be possible to splice genes from one organism into another and between animals, plants, and microorganisms. This idea was realized using enzymes that cut DNA threads (restrictuon endonucleases) and others that splice the cut ends together (DNA ligases). In general, restriction endonucleases make staggered cuts at positions in a double-stranded DNA thread where the letters form short palindromes on opposite threads, e.g., the enzyme *Eco* Ri would cut as follows,


cut
G T G C G A A T T C T C T
C A C G C T T A A G A G A
cut
opposite-strand palindromes

leaving one thread on each side of the break entending beyond the other as above and in figure 3A. Although both threads (polymers of repeating sugar-phosphate molecules) are cut apart, the two parts tend to separate slowly because of stickiness between the As and Ts on opposite threads. When two different cut threads are brought together (as are the gene and plasmid in figure 3A), the staggered ends intermix and stick together causing some of the genes to be inserted into plasmids forming rDNA plasmids. A DNA ligase is then added to splice the sugar-phosphate polymer threads together. Two methods for reading the sequence of the letters T, C, A, and G along DNA and spliced DNA threads have been invaluable to the rapid development of rDNA technology. One of the methods permits a single worker to determine the sequence of 400 to 500 letters in a day's time, and the method is presently being automated to further reduce the human input.

Applications of genetic engineering -- including rDNA, monoclonal antibody, embryo transfer and allied procedures -- began to expand rapidly by the late 1970s. Since then, upwards of 300 companies, both newly formed and established chemical and pharmaceutical firms, in Western Europe, Israel, Japan, and the United States have invested heavily in the commercial development of this technology. The impetus for this intense competition was the anticipation of the returns to be expected from the marketing of products and processes, not previously available, of great benefit for humans and the production of plants and animals. In the United States alone, owing to these expected benefits and favorable investment and tax climates, a few billion dollars of venture capital have been raised to support research and development in genetic engineering.

Five interrelated lines of research and development in animals will be covered in this paper: 1) production of pharmaceuticals, 2) control of infectious diseases, 3) improvements in animal production, 4) detection and treatment of genetic abnormalities, and 5) elucidation of the molecular mechanisms of cancer and leukemias. Gene cloning and monoclonal antibody techniques are used in all five lines; the third line also involves embryo manipulations.

Three basic genetic engineering techniques are shown in figure 3. In rDNA procedures (figure 3A), a foreign gene is spliced into a carrier DNA (called a vector) for insertion into a new microorganism or animal host, and is made to replicate (called cloning a gene) and produce (called express) its protein product, e.g., an enzyme, hormone, interferon molecules or surface protein of an infectious agent. Monoclonal antibodies (figure 3B) are usually produced by fusing an antibody-producing cell (e.g., a spleen cell of an immunized animal) with a malignant myeloma cell, generally from the same animal species. The fused cell, called a hybridoma, has the perpetual growth characteristics of the myeloma cell and the spleen cell's ability to produce multiple copies of a single species of antibody molecule reactive with a specific site on the antigen used to immunize the animal. Many differenet monoclonal antibodies can be produced by this fusion technique because each spleen cell produces only one kind of the many species of antibody that can be induced by an antigen. Embryo manipulations (figure 3C) include artificial transfers of embryos from the donor animal to surrogate mothers. Before transfer, the isolated embryos may be treated in a variety of ways, including splittings, fusions with other embryos and injection with foreign genes.

Current State of the Art

Genetic engineering has emerged with remarkable rapidity as the dominant new technology in biological research and development. Advances using gene transplantation, monoclonal antibodies and embryo manipulations can be compared with advances in the computer/electronics industry that have been made with transistors and microchip circuitry. In fact, the two technologies are beginning to merge: computers are being developed in which silicon microcircuit chips are being replaced by protein biochips produced using recombinant-DNA procedures. Some eager advocates suggest that such "biochip computers might be able to design and repair themselves, evolve, show emotions and serve as the basis for true artificial intelligence" (Baum, 1983). However much this view resembles science fiction, even a realistic look at where genetic engineering is today portends a bright future for improvements in animal health and productivity, even in the absence of additional fundamental breakthroughs. The following survey is an overview of the present status of genetic engineering in each of the five research and development lines listed in the preceding section. It should be remembered that the lines are interrelated and that human models are included even when comparable developments for animals are lacking.

Production of Pharmaceuticals

A major thrust of genetic engineering has been the mass production in microorganisms of proteinaceous pharmaceuticals, including a number of hormones, enzymes, activating factors, amino acids and feed supplements. Certain of these biologicals were previously obtained only from animal and human organs, and consequently were either unavailable in practical amounts or in short supply and costly. The first production of a hormone, somatostatin, by genetic engineering in 1977 was described by the late Dr. Philip Handler in testimony before Congress (Schmeck, 1984) as "a scientific triumph of the first order." In this case, even the gene for somatostatin had to be synthesized artificially prior to its insertion into the host bacterium. Thereafter, gene splicing progressed rapidly, and by 1982 the FDA approved human insulin as the first genetically engineered pharmaceutical for general use in the United States. An impressive number of additional pharmaceuticals and nutrients that have been genetically engineered are listed in table 1. The list is no doubt incomplete owing to the rapidity of advances in both the U.S. and abroad and to secrecy required to protect proprietary interests. Many of the genetically engineered biologicals in table 1 are for human applications. However, as required for improving animal health and productivity, analogous animal products are appearing, for example, bGH, pGH, cGH and rennin (Seeburg, 1983); and a hormone releasing factor is under development (Sella, 1983). The recent announcement of genetically engineered hCG (GEN, v4/1, 1984) will surely be followed by bCG for application in superovulation and embryo transfers in bovines. Presumably, bovine prolactin when expressed from its cloned gene (table 1) will be given to pregnant cows before calving to establish higher than normal levels of lactation. Several firms including Eli Lilly (Time, 1984) are developing genetically engineered bGH to stimulate lactation in cows. In trials at Cornell University, daily doses of such recombinant bGH increased the milk production of cows by about 12 percent, and an industrial-scale delivery system for the hormone is reportedly being developed by Monsanto (GEN,v2/5, 1982). Meanwhile, an experiment has been reported that has doubled the heritable size of mice by insertion of hGH genes into the germplasm of mice (Palmiter, 1983), indicating that analogous insertion into the bovine germline of additional bGH genes or of growth hormone genes from large mammals such as sperm whales or elephants could yield larger productivity gains than somatic injections of growth hormones and, in addition, remain as a permanently inheritable characteristic. The expression "a whale of an animal" would no longer be just a figure of speech. It is probable, however, that the copy number of genes in a cell is crucial to growth, and that increasing that number with homologous GH genes will suffice. The first transgenic rabbits, sheep, and pigs carrying hGH genes did not evidence growth enhancement (Hammer, 1985). Perhaps, expression of the hGH genes was inadequate in these trials or the cell receptors for growth hormone in these animals are highly species-specific. If the bioengineered cGH which is active in rats, can produce a 15 percent or greater enhancement of growth in chickens (BN,v3/4, 1983; GEN,v4/1, 1984), it could be of economic value to the poultry industry provided that mass injection methods are developed or that germline insertion of the genes can be accomplished. The cloned porcine relaxin (Stewart, 1982), produced naturally in ovaries during pregnancy, could be of use in problem piglet births, but an analogous bovine product (not yet reported) would seem to be of greater application for easing difficult births in Charolais and other bovine breeds. The reportedly tight supply of natural rennin, from the fourth stomach of suckling calves, used for making cheese could be relieved by the genetically engineered enzyme, made in both bacteria and yeast (C&EN, 1982). Rennin is the first enzyme of industrial importance to be cloned and should be on the market by the mid-1980's. Bioengineered cellulases, which degrade cellulose to sugars, have been produced by programming Escherichia coli bacteria with both normal and thermophilic cellulase genes (C&EN, E. coli..., 1983). Cellulase from the latter gene still functions at 65°C, a temperature that helps rid the E. coli culture of potentially contaminating microorganisms. While the bioengineered normal and high temperature cellulases are expected to be used for degrading cellulosic plant wastes to sugars, analogous reprogramming of rumen microorganisms could increase the utilization by ruminants of higher cellulosic feeds and of marginal grasses and woody plants. The bioengineered amino acids and antibiotics in table 1 will be useful for both animals and humans. The single-cell proteins are being introduced as animal and poultry feed additives. However, Probion, which has been processed to remove phospholipids and nucleic acids, is said by its manufacturer to be suitable for use in human foods; and work at MIT is directed toward processing Provesteen into a similar product (BN, 1982, C&EN, Single..., 1983). The U.S.S.R., owing to its plentiful supply of methanol, which is feedstock for single-cell protein, has recently expressed interest in licensing the U.K. Imperial Chemical Industries (ICI) Pruteen technology (C&EN, U.S.S.R...., 1984).

Control of Infectious Diseases

Recombinant DNA and hybridoma methodologies are being used for the detection, prevention, and treatment of infectious and genetic diseases. The former methodology is providing 1) cloned DNA sequences for use in the diagnosis and treatment of diseases, 2) cloned surface proteins of infectious agents for use as vaccines, and 3) cloned immune modulators such as interferons and interleukin-2, Hybridomas are providing highly-specific monoclonal antibodies (see Introduction) for use in diagnosis, passive immunization and treatment of diseases.

DNA Probes

A wide variety of cloned DNA sequences are becoming available for use as probes in both human and veterinary medicine. A DNA probe, coupled to an appropriate enzyme, indicates by a color change or by chemiluminescence when it has hybridized to complementary DNA in the diagnostic sample. Radioactively-tagged probes as used in basic research are not suitable for routine clinical and field use. Nonisotopic probes are being developed to detect *Salmonella*, *Shigella*, and *Campylobacter* poisoning in meats and other foods. The probe for *Salmonella* is slated for marketing in 1984 (GEN,v4/1, 1984). Others are being developed for gonorrhea, cytomeglia, herpes, hepatitis B, bluetongue and canine distemper infections and for the presence of enterotoxigenic *E. coli* in piglet, calf, and human diarrheas. Probes are also under development for prenatal and post-natal histocompatibility antigen typing, and for the early detection of cystic fibrosis genes, cancer oncogenes and genetic mutations caused by carcinogens. The most advanced application of DNA probes will be for therapy, making it possible to control the activity of specific genes. The NIH's rationale for approving the cloning of diphtheria toxin (table 1) was for its possible usefulness (coupled perhaps to a DNA probe) in destroying cancer cells such as malignant melanomas (NYT, Gene-Splicing..., 1983; Leong, 1983). While probes are finding their greatest early use in human medicine, a lowering of costs will make them available for animal health care.

Cloned Protein Vaccines

The production of cloned surface proteins of infectious agents for many important animal and human diseases is a very active field of research. A vaccine of this type for foot-and-mouth disease, produced in 1981, was described by Secretary of Agriculture John R. Block "as the first effective vaccine produced by gene splicing for any disease of animals or humans." Table 2 lists a number of cloned protein vaccines under development (Bachrach, 1982; Bachrach, 1983). Protein vaccines have potential advantages not possessed by conventional attenuated or killed virus vaccines. Not being derived from virus particles, they cannot cause infection and are less prone to produce side effects than virus vaccines. Being proteins, they can also be freeze-dried and stored without refrigeration, which is an important advantage in underdeveloped countries and elsewhere where refrigeration is lacking. Most of the cloned protein vaccines, however, are still in various stages of development and will require extensive field testing before being approved for general use.

Cloned protein vaccines have been developed for neonatal scours in livestock caused by enterotoxigenic _E. coli_. These bacteria produce diarrhea by adhering with their pili to the intestinal wall, colonizing the wall and secreting a toxin. A vaccine produced in Holland and in use in Europe consists of the cloned pili protein. Another vaccine produced in the United

States by rDNA technology has been approved by the USDA for use in swine (GEN,v3/3, 1983). It contains one antigen that stimulates the production of an antitoxin and three others that produce antibodies that block adherence and colonization by the bacteria. The vaccine is administered to pregnant sows shortly before parturition so that the piglets will acquire immunity through the sow's milk. Cloned protein vaccines in the early stages of development include those for avian coccidiosis (USDA, Notes..., 1983) and malaria (Ellis, 1983). Immunogenic porin proteins from Brucella abortus have been identified (Winter, 1983), but their genes have not been cloned.

Other Approaches to Vaccines

Successes with cloned protein vaccines and monoclonal antibody methodology have produced additional approaches to vaccines:

1) organic synthesis of short peptides containing only the immunogenic sites of the surface proteins of infectious agents,
2) live recombinant viruses, most commonly vaccinia virus recombinants, having guest genes for the surface proteins of other infectious agents,
3) genetically engineered live nonreverting deletion mutants of infectious agents, and
4) anti-idiotype antibodies (also called antibody-2) as vaccines. Antibody-2 is made to antibody-1 and consequently mimics the surface of the original antigen.

Experimental vaccinations have been carried out in method 1 with short chemically-synthesized peptides for foot-and-mouth disease, polio, influenza, herpes, hepatitis B, vesicular stomatitis, diphtheria, and cholera (Bachrach, 1983; Cohen, 1984; Dreesman, 1982, Yilma, personal communication; Audibert, 1982); in method 2 with recombinant vaccinia viruses for hepatitis B, herpes and influenza (Smith, Infectious..., 1983; Smith, construction..., 1983) as well as for transmissible gastroenteritis of swine, rabies, vesicular stomatitis, Friend murine leukemia, and malaria (Bachrach, references *in press*); in method 3 with nonreverting mutant viruses for herpes and Salmonellosis (Roizman, 1982; Smith, in press); and in method 4 with anti-idiotype antibodies for hepatitis B and rabies (Kennedy, 1983; Regan, 1983) as well as with polio, reovirus and bacterial infections, trypanosomiasis, and cancer antigens (Bachrach, references *in press*).

The recombinant method using vaccinia virus as the vector for guest genes appears to have considerable potential. Only a small quantity of the live recombinant is required for vaccination, because replication of the recombinant virus expresses the surface proteins coded for by the guest genes. Moreover, vaccinia virus can act as host to several guest genes in tandem, thereby having the potential of producing immunity to many diseases concurrently. In addition to the three recombinants already tested, a recombinant for malaria is under construction (Moss, personal communication). However, it is known that vaccinia virus, although still used for vaccinating U.S. military recruits against smallpox, produces disseminated vaccinitis in about one in every 300,000 vaccines with a 20 percent mortality in those so affected (Moss, personal communication). It is not known, however, whether the recombinant vaccinia viruses produce this or other adverse reactions.

The usefulness of genetically engineered nonreverting mutant agents has not been thoroughly explored. In general, two or more genes have been engineered out of these agents. In contrast, the commonly used avirulent agents that are obtained by selection alone can occasionally revert to virulence, as occurs with the Sabin oral poliovirus vaccine strains (Kew, 1981).

Reports of tests of anti-idiotype antibodies as vaccines and anamnestic sensitizers of the immune response are increasing, because these antibodies can now be produced in large quantities in hybridomas.

Genetically Engineered Immune Molecules

Monoclonal antibodies and other protein molecules of the immune system collectively called lymphokines and cytokines are being produced by genetic engineering techniques for use in human and veterinary medicine.

Monoclonal Antibodies

Monoclonal antibodies, as described above, are generally produced in hybridomas formed by the fusion of antibody-producing cells and myeloma cells (figure 3B). However, two additional routes to monoclonal antibodies have been reported recently. In one, antibody-producing cells are transfected with DNA from leukemia or myeloma cells (SN, 1983); in the other, DNA transcribed from purified messenger RNA molecules that code for antibody molecules is cloned in microorganisms (GEN,v3/4, 1983), as in figure 3A. Hybridomas can, in theory, be grown indefinitely producing large quantities of monoclonal antibodies, for example, 0.1 mg/ml from _in vitro_ hybridoma cultures and 10 mg/ml from the ascitic fluid of hybridoma-bearing mice. Also, hybridomas can be frozen in liquid nitrogen for regrowth at future times.

The many important uses of monoclonal antibodies (table 3) far outweigh a few limitations, one of them being that their high degree of specificity may make them less useful than polyclonal antibodies in initial screenings for diseases that have many serotypes. Their use as vaccines has been described above. Bonded to gel columns (called affinity columns), monoclonal antibodies are widely used in laboratory and production-scale work to purify antigens from highly complex mixtures, including valuable products from genetically engineered microorganisms. Monoclonal antibodies are powerful tools for unravelling the complexities of the immune network in animals, for example, in differentiating white blood T-cell (T-lymphocyte) subpopulations and their lymphokine products, and in characterizing the major histocompatibility complex of antigens (including transplantation antigens) more precisely than was possible with polyclonal antibodies. A monoclonal-based procedure for sexing livestock embryos has been reported which has greater than 90 percent accuracy (GEN,v4/1, 1984). Monoclonal antibodies are also used to identify antigenic, immunogenic and pathogenic sites on proteins, infectious agents and cells, as well as for recognizing mutations expressed as changes in phenotype. Influenza, herpes, rabies, adeno, foot-and-mouth, parvo (cat, canine, and porcine) and bluetongue viruses and malarial and trypanosomal parasites are a few of the infectious agents of animals that are being characterized by monoclonal antibodies. For trypanosomes, in particular, monoclonal antibodies are defining the large number (40-100) of antigens that arise during developmental cycles of the organism and in response to the immune system of the host animal.

The promise of monoclonal antibodies for use in disease control has been a principal factor in the start up, over the past 4 or 5 years, of many of the two hundred and more new genetic engineering enterprises. The goal is to develop monoclonal antibodies for the diagnosis, prevention and treatment of cancer and the major infectious diseases of animals and humans. Monoclonal antibodies to the pili of enterotoxigenic _E. coli_ have undergone successful field tests in Canada for the prevention of scours in calves, and have been approved by the USDA for use in the United States (GEN,v4/1, 1984). It has been suggested that monoclonal antibodies to parainfluenza-3 virus could help control shipping fever in cattle (GEN,v4/1, 1984). Monoclonal antibodies are being developed against parasites that cause coccidiosis in chickens, a disease that costs the poultry industry about 300 million dollars a year. The antibodies should be useful for diagnosing these parasites in chickens or whether an immunity to the disease has developed (ICN, 1982). Monoclonal antivenoms are being tested as replacements for antivenoms conventionally produced in rabbits and horses. Monoclonal antivenoms to the lipase-like component of the deadly venom of the brown recluse spider is reported to be superior to antivenom produced in rabbits (GEN,v3/2, 1983). Nevertheless, monoclonal antibodies are not yet magic bullets to treat cancer, although that goal is still envisioned (Kolata, 1983; ICN, 1984). However, patient-specific monoclonal antibodies have been reported to induce remission of B-cell lymphomas (Miller, 1982; GEN,v3/6, 1983). Also, one company is awaiting FDA approval of monoclonal antibody diagnostic tests for intestinal and ovarian cancer, and another is readying monoclonal antibody for measuring the level of a drug used to treat asthma. Despite their high specificity, monoclonal antibodies do not at present differentiate accurately enough between benign and malignant tumors, and consequently are best suited now for monitoring the progress of known cancerous conditions.

Lymphokines and Cytokines

Lymphokines produced by white blood cells and cytokines produced by other cells help to regulate the immune defenses of animals to foreign antigens, infectious agents, cancer and autoimmune disease. While there may be more than one-hundred such substances, only a few have been isolated and purified in amounts required for determination of their structures and precise functions. Even fewer lymphokine and cytokine genes have been cloned and expressed in microorganisms (table 4).

The alpha, beta, and gamma classes of interferons are produced, respectively, in leukocytes, fibrocytes/epitheliocytes and lymphocytes. The alpha and beta classes are induced in response to viruses, polyribonucleotides and bacterial products, whereas the gamma class, also called immune interferon, is induced by antigens, antigen-antibody complexes, antilymphocyte serum and mitogens. Currently, five groups are involved in producing cloned gamma interferon for trials and marketing. The hybrid class has been artificially produced by gene splicing. Called consensus interferon, it is a composite of 13 subtypes of human alpha interferon (GEN,v4/1, 1984). Interferons, when first discovered in the late 1950s, appeared to be animal-species specific, for example, interferon produced in bovine cells would only protect bovine cells but not chicken cells or cells of other animal species against viral infection, and vice versa. This property is now known to vary with both the animal species and class of interferon. Thus, some human alpha interferons protect nonhuman cells, whereas human beta interferons will not. The artificial hybrid interferons have relatively high activities in human, bovine and murine cells. Prior to the production of the recombinant interferons, the quantity of the natural interferons was insufficient for other than limited clinical testing, and more often than not the results were inconclusive owing to impurities present in the samples. In contrast, genetically engineered interferon is virtually pure and available in quantities sufficient for well-controlled clinical trials against immune disorders, infectious diseases and solid neoplasms and leukemias. While many of the cancer trials have not met with original expectations, very recent results of treatment of hairy cell leukemia with cloned alpha interferon are indeed promising (Gutterman, TV commun., 1984).

Alpha, beta and gamma bovine interferons and hybrid interferon will benefit veterinary medicine for treating valuable breeding stock, race horses and show and companion animals. Because of curious jurisdictions, the FDA rather than the USDA will regulate bovine interferons (GEN,v3/6, 1983). Since interferon ordinarily has to be inoculated daily, there is a need for slowly-dissolvable injectable carriers impregnated with interferons. Intranasal instillation of cloned human alpha interferon, however, protects calves against challenge with bovine rhinotracheitis virus (Gustavson, personal communication), and consequently may be useful in preparing animals for shipment. Also, interferon genes are expected to be among the first selected for insertion into the germplasm of animals (see following section).

Interleukin-2 (IL-2) is an important lymphokine that has been cloned and expressed in microorganisms, first in Japan (BN,v3/4, 1983). This lymphokine promotes the growth of helper T-cells, i.e., T-lymphocytes, that enhance antibody production. In normal individuals the ratio of helper T-cells to T-cells that suppress antibody production is about 2 to 1; in immunosuppressed individuals, e.g., AIDS patients, the ratio reverses to about 1 to 2. The addition of IL-2 to _in vitro_ cultures of T-cells taken from AIDS patients markedly increases the helper to suppressor T-cell ratio, and tests have been initiated to determine if a similar effect accompanied by patient improvement will occur _in vivo_ (GEN,v4/1, 1984). If effective, the animal equivalent of human IL-2 may be similarly useful in treating immune deficiency diseases of animals.

While lymphokines, hormones and enzymes are being produced in microorganisms, post-translational modifications (e.g., specific protease cleavages, glycosylations and secretion from the cell) needed to activate many proteins are best carried out by animal cells. Animal cells, however, generally require significant amounts of costly serum or lymph for growth. One innovative company has devised a unique method of providing large volumes of lymph using a herd of 18 Holstein high-tech cows retired from dairies (Eckhouse, 1984). In a semi-closed system, lymph drawn with catheters from the cows' thoracic arteries is circulated through porous hollow fibers immersed in animal cell growth vessels. Nutrients from the lymph pass through the pores of the fibers into the cell growth medium. Without apparent harm, one cow can push 200 liters of lymph per day through the growth vessel and receive 80 percent of it back. Natural and reprogrammed animal cells that produce interferons, insulin, monoclonal antibodies and a blood-clotting factor are being mass produced in this system.

Improvements in Animal Production

Advances in reproductive biology and their effects on animal improvements are described in considerable detail in a previous publication of the OTA (Impacts..., 1981), in a companion paper in the present study by Hansel, and in a paper on cattle by Seidel, 1981. Therefore, this discussion will be concerned primarily with germline insertions of heterologous genes into animals and proteciton of animals against ectoparasites by the sexual sterilization of insects.

Embryo Transfer and Gene Insertions

A new technique arising from the convergence of gene and embryo manipulations promises to permit genes for new traits to be inserted into the germlines of livestock and poultry. It opens a new world of possibilities for improvements in animal health and productivity, heretofore only present in fanciful dreams and science fiction. Unlike the gain in somatic productivity that an animal makes from injections with a bioengineered growth hormone, animals of the future will be endowed permanently with traits of other animals and humans, and probably also of plants. In the new technique, genes for a desired trait are injected directly into either of the two pronuclei of a fertilized ovum. Greater success rates have been reported, however, by injection into male pronuclei. Upon fusion of the pronuclei, the guest genes become a part of all of the cells of the developing animal and are, moreover, transmitted to succeeding generations. Successful insertion of foreign genes into the germline of mice has been demonstrated repeatedly, and applications to livestock and poultry are underway. Mice have been endowed with genes for rabbit beta globin, a viral thymidine kinase enzyme, and antibody, interferon, insulin and rat and human growth hormones (Schmeck, 1983). Mice endowed with the gene for human growth hormone grow twice normal size.

Embryo transfer in cows including the modification required for gene insertion is depicted in figure 3C. A superovulated donor animal (see companion paper by Hansel for details) is artificially inseminated, and the resulting embryos are removed nonsurgically for implantation in and carrying to term by surrogate mothers. Prior to implantation, the embryos can be treated in a number of ways. They can be sexed, split (generally to make twins), fused with embryos of other animal species (to make chimeric animals or to permit the heterologous species to carry the embryo to term) or frozen in liquid nitrogen. Freezing is of great practical importance because it permits embryos to be stored until estrus of the intended recipient on the farm is in synchrony with that of the donor. For gene insertions, the embryo must be in the single-cell stage having pronuclei that can be injected with cloned foreign genes, as described above. The genes likely to be inserted into cattle may be those for growth hormones, prolactins, digestive enzymes and interferons, thereby providing both productivity gains and enhanced resistance to diseases. Gene insertions may be the only means of preventing diseases that present so many surface antigens that vaccine technology is difficult or impossible. For example, if the trypanotolerance of certain strains of cattle and sheep resides in a few distinct genes, it may be possible to insert the cloned genes into the germplasm of livestock in trypanosomiasis-affected regions of the world.

Whole-body cloning of animals (frogs and mice) has also been achieved. Both pronuclei of a fertilized ovum are replaced with pronuclei or an embryonic nucleus of the same species (McGrath, 1983). For frogs, a tadpole nucleus can be used, but the developing form will terminate at this stage also. If whole-body cloning can be adapted to large animals using pronuclei or nuclei of related species, the procedure would be useful for preserving rare species and, possibly also, for reviving extinct species from cryopreserved gonadal cells or embryos (GEN,v3/6, 1983). While such feats may seem like science fiction, the power of genetic engineering is bringing them into the realm of the possible. The genes of endangered and revived species could someday prove valuable in engineering improvements in livestock and poultry.

Table 5 lists some achieved and anticipated improvements in animals from embryo and gene manipulation procedures. While less than 1 percent of U.S. cattle are involved in embryo transfers, the obvious benefits will push the percentage upward rapidly, particularly as the costs of the procedure decrease (Brotman, 1983). One company, GEI, already markets frozen cattle embryo both domestically and abroad, and provides an embryo sexing service for cattle breeders (GEN,v3/6, 1983). Embryo transfer will have an especially great impact in areas of the world where animal and poultry production have been marginal. Application is expected to be most successful in countries with existing infrastructures in animal husbandry and veterinary medicine. Long used in cattle, embryo transfer is beginning to gain acceptance in mitigating infertility problems in the human population. The first human birth from a surrogate mother implanted nonsurgically with an embryo from a donor occurred in January of 1984 (NYT, 1984).

Protection Against Ectoparasites

No account of genetic manipulation for the betterment of livestock would be complete without mention of the USDA's success in protecting cattle against ectoparasites by the sexual sterilization of insects. The most notable use of this technique has been in the eradication of the cattle screwworm from Florida, the Southwest and in a barrier zone in Mexico south of the U.S. border. In this procedure, the pupae of as many as 100 million screwworm flies are raised per week, sexually sterilized by irradiation with cobalt-60, packaged a few hundred to a lot and dropped from the air over infested areas. When the sterile flies mate with endemic flies, no offspring are produced, thereby eradicating the species.

Detection and Treatment of Genetic Abnormalities

Genetic engineering has developed DNA probes and restriction endonuclease tests to identify and locate genes responsible for inherited diseases, and is devising procedures for therapy. The DNA probes bind to the point(s) of defectiveness in the genes; and restriction endonucleases, which cut DNA at specific points, produce DNA fragments that are diagnostic of a heritable defect. The DNA probe method detects misspellings, often one wrong DNA letter, that are responsible for defectiveness in genes, thus identifying individuals and fetuses that are at risk of developing inherited diseases long before their onset. Cystic fibrosis is the most common of these diseases in humans. Others are muscular dystrophy, Huntington's chorea (a nervous disorder), sickle-cell anemia, beta thalassemia, hemophilia, emphysema and arteriosclerosis (defects in several genes) (McAuliffe, 1983). While genetic diagnosis is used principally in humans, comparable applications exist in veterinary medicine, for example, in examining fetal cells removed by amniocentesis for detecting abnormalities in the fetuses of prize breeding stock.

By comparison, gene therapy is a much more futuristic technology. However, like embryo transfer and gene insertion methods, gene therapy is likely to score most of its early successes in animals, owing to the ethical issues involved in treating humans (NYT, 1982; Davis, 1983). Actually, germline gene insertion will likely be an important strategy in achieving effective gene therapy. Another strategy will be gene replacement. It was attempted without prior NIH approval and without success: bone marrow cells removed from beta thalassemia patients were exposed to normal genes for hemoglobin and reimplanted into the bones of the patients. A third strategy, tried with some success in patients with beta thalassemia and sickle-cell anemia, involved the use of the drug 5-azacytidine to reactivate the patients' fetal hemoglobin genes (probably by removal of methyl groups) that are normally turned off shortly before birth. The anemias were markedly reduced for the one week of treatment, with some of the effect lasting for up to a month; however, prolonged use of this drug is contraindicated because of the risk of its causing cancer (Schmeck, Activity..., 1982). Another approach to beta thalassemia therapy involves forcing the defective mRNA for beta globin to be translated through an internal termination codon UAG mutated from the normal AAG for lysine (see figure 2). This has been accomplished in frog egg cells by inserting a gene for a transfer RNA tailored to translate the internal UAG as a codon for lysine and thus complete the beta globin chain (Schmeck, Researchers..., 1982).

Control of Cancer and Leukemias

Recombinant DNA procedures and monoclonal antibody probes are contributing importantly to rapid progress in elucidating the molecular mechanisms of cancer. Studies on the reproduction of certain cancer producing RNA viruses, called retroviruses, led to the conclusion that proto-cancer genes (called proto-oncogenes) are present in the cells of drosophila, birds, animals, and humans. Being so widespread and preserved throughout evolution, the proto-oncogenes are thought to have functions necessary for embryonic development, but to be turned off at birth. The linkage between embryogenesis and carcinogenesis is supported by the observations that 1) an antigen, called carcinoembryonic antigen, similar to one produced in embryos is also produced by tumor cells and 2) a set of genes in transformed mouse cells share repetitive DNA sequences with genes transcribed in mouse embryos (C&EN, Some..., 1984).

When a proto-oncogene is activated by environmental carcinogens, it becomes an active oncogene responsible for the production of certain proteins that promote abnormal (cancerous) cell growth. Thus, the development of cancer requires that inherited genes be mutated by environmental carcinogens. Some known carcinogens include halogenated hydrocarbons and other alkylating agents; polycyclic hydrocarbons such as benzopyrene in cigarette smoke; some food preservatives (e.g., nitrites which give rise to nitroso compounds; pesticides like ethylene dibromide; overly charred meat; fungal aflatoxins; excessive X, gamma or ultraviolet radiation; and many classes of viruses (papova, herpes-2, retroviruses, hepatitis B and some adenoviruses). Of these, certain retroviruses, polycyclic hydrocarbons and nitroso compounds (Sukumar, 1984) are known activators of proto-oncogenes. A buffer against cancer on which there is general agreement, however, is that the clinical disease develops in several steps, never less than two, and that any one of the many cancer genes in animals and humans probably contributes to only one of the steps. Thus, it may well be that initial changes leading to cancer occur quite frequently, but that the body's immunological surveillance almost as frequently destroys the cells so affected before they are fully transformed and attain a critical mass needed to perpetuate uncontrolled growth and metastases.

The molecular mechanisms of cancer have come largely from studying retroviruses, some of which have acquired oncogenes from the cells they infect, owing to the unique method by which retroviruses replicate. Retrovirus RNA is transcribed into DNA in the cytoplasm, and the DNA migrates into the nucleus where it integrates (becoming proviral DNA) into the cellular DNA genes (it should be observed that these steps are just the reverse of those shown in figure 1). Subsequent to certain stimuli, this integrated proviral DNA is transcribed into retroviral RNA that, in turn, is translated into viral proteins for assembly together with the viral RNA into virus particles (these steps are in the same direction as those shown in figure 1). The transcription of the integrated proviral DNA into viral RNA may sometimes also include transcription of a cellular oncogene, thereby transferring oncogenicity to the retroviral RNA.

At least 17 proto-oncogenes have been identified in lung, bladder and colon-rectal human tumors; they are located on chromosomes 1-9, 11, 12, 15, 17, 20, 22 and X (D'Eustachio, 1984). Several connections have been established between oncogenes and cancer. One connection is the translocation of genes between chromosomes: in one example, an oncogene called myc translocates from chromosome 8 to chromosome 14 where it can interfere with the production of antibodies. Furthermore, there appears to be a linkage between translocation and amplification (increase in gene copy number) of myc and possibly of other oncogenes. In other connections, cellular oncogenes have been found to code for proteins that regulate cell growth: a src gene for a protein kinase that phosphorylates tyrosine; a sis gene for platelet-derived growth factor (C&EN, Virus-made..., 1983); and infection of cells having an erb-B gene with chicken leukemia virus produces a defective cell receptor that induces abnormal cell growth without activation by epidermal growth factor (EGF) (C&EN, Second..., 1984). In addition, in certain bladder carcinomas point mutations causing replacement of glycine (lacking a side chain) with a side-chain amino acid such as valine, serine, arginine or lysine in position twelve of proto-oncogene-encoded p21 protein appears to alter the protein's structure and implicate it in carcinogenesis (GEN,v3/2, 1983). Also, the transcriptional control sequence of the proviral DNA of a retrovirus, called the long-terminal repeat (LTR), enhances cell transformation when integrated on either side of a cellular oncogene (Miller, 1982; Wood, 1983). To summarize, oncogene-carcinogenesis connections include: oncogene translocations and amplifications; encodings for a tyrosinyl protein kinase, cell growth factors and a constitutively-active cell growth receptor; structurally-defective p21 proteins; and LTR enhancement.

Advances in cancer and leukemia research using rDNA methods and monoclonal antibodies, carried out principally in cell cultures, mice and human tissues, should eventually help to control neoplastic diseases in livestock and poultry. Two important diseases of animals caused by retroviruses are bovine leukosis and equine infectious anemia. Based on success with a vaccine for Friend's mouse leukemia composed of the envelope glycoprotein of the virus, called gp71, work is now in progress toward developing a vaccine for bovine leukosis, which would consist of the cloned outer protein of the virus, gp52. Conventional control of the disease consists of the elimination of leukosis positive animals from breeding stock. Marek's disease of chickens is especially notable because it is the only neoplasm that has been controlled by vaccination. The vaccine is live turkey herpesvirus. This cross protection is considered due to similarities in the glycoproteins A and B of these two herpesviruses (Glaubiger, 1983). A high degree of hybridization between the DNAs of these viruses tends to support this conclusion (Nazerian, personal commun.). Breakthroughs in cancer research are also needed to gain more effective control of the avian leukosis group of poultry diseases caused by retroviruses.

MAJOR BREAKTHROUGHS NEEDED

Obstacles

Genetic engineering enterprises are confronted with few obstacles that will not be overcome. For example, a needed breakthrough in rDNA technology, to increase the yield of the cloned protein, has recently been achieved. Generally, transformed bacteria maintain a low copy number of their rDNA plasmids, e.g., 2-20 per cell (figure 3A), but a new advance, called runaway plasmid replication developed in Denmark, pushes the copy number above 1,000, thereby increasing the yield of protein by 50 to 500 fold (GEN,v3/3, 1983).

Also, while almost any gene manipulation imaginable can be carried out on a laboratory scale, certain problems arise in scaling them up to the size required for commercialization. Large-scale purification of a cloned protein from a cell (yeast, bacterial or animal) is expensive. Monoclonal antibody affinity columns and engineering the protein for secretion are two methods to alleviate this difficulty. While extraction of the protein can be accomplished by enzymatic and mechanical disruption of the cell wall, it is preferable to provide the desired protein with the characteristics of secretory proteins, provided that such modifications do not alter the protein's biological activity. Fortunately, this methodology is now being developed.

Certain proteins that require post-translational processing, such as glycosylations and removal of hydrophobic anchor sequences to permit secretion, are best cloned in animal cells. However, the large-scale fermentation of animal cells is very costly owing to the large amounts of animal serums required in the growth medium. So-called serum-free mediums for animal cells have never really been of practical use. However, the innovative use of lymph circulated from high-tech cows (see Lymphokines and Cytokines) shows great promise for circumventing this problem.

Another solution may come from the new recombinant RNA (rRNA) technology (GEN,v4/1, 1984). When mRNA coding for a desired protein is spliced, in the test tube, to the transcription-initiating end of the RNA of a bacterial virus called Qβ and purified Qβ replicase is added, millions of copies of the rRNA are synthesized. This rRNA can serve as mRNA in cell-free and cellular protein synthesizing systems or can be reversely transcribed into DNA for cloning into microorganisms as shown in figure 3A. A breakthrough to an efficient cell-free translation system for _r_RNA could cause a shift away from rDNA to _r_RNA technology.

Problems could develop for the new vaccinia recombinant vaccines for several diseases (e.g., hepatitis B, herpes and influenza) if their use results in instances of disseminated vaccinitis. There is also the need to sharpen the specificity of monoclonal antibodies so that they image and target benign and malignant tumors selectively. Also, the efficacy of superovulation in donor cows needs to be increased to provide more embryos, thereby lowering the cost of the embryo transfer procedure.

Funding

There appears to be no end to the ability to raise venture capital and capital from established pharmaceutical and chemical industries to fund genetic engineering enterprises in the United States. How else did some 200 or more genetic engineering laboratories spring from nowhere in the last few years? This unusual phenomenon has two major causes: 1) the great benefits arising from the revolutionary field of genetic engineering and 2) a tax climate in the U.S. highly favorable to venture capital investment.

Institutional Arrangements

Genetic engineering is being conducted by all elements of the research community -- by Federal and State governments, public and private universities, and private industries. In several instances, information developed in Federal laboratories has been rapidly transferred to industry for commercial development. New York State has set up an organization to capitalize on findings of its research laboratories. In one case, Federal and private industry scientists pooled their talents to develop a vaccine for foot-and-mouth disease by rDNA technology. The NIH appears to have adjusted its research programs to accommodate and capitalize on the new genetic engineering technology. Moreover, it further promotes genetic engineering research through its grants programs and by contracting out certain phases, such as a basic cancer research program at Frederick, Maryland. In contrast, the U.S. Department of Agriculture's commitment to genetic engineering in animals has been quite limited. Presidential Science Advisor George Keyworth stated at a National Academy of Sciences meeting on genetic engineering in May of 1983 (GEN,v3/4, 1983) that "the leaders of agriculture research are not familiar enough with recent advances in molecular biology, or are too comfortable with traditional approaches, to be able to exploit these new opportunities." Part of the problem may be that there is no molecular biologist in the top echelons of the program staff of the USDA to help promote innovative approaches to genetic engineering research in animals. Perhaps, the recent USDA/ARS reorganization plan will help to redress these deficiencies, but too little funding appears to have been committed to basic genetic engineering in animals (Marshall, 1983). In the private sector, many industries have made very substantial grants to universities and medical research centers for genetic engineering research, usually with the right of first refusal to license products having commercial potential. To name a few, Hoechst AG has guaranteed at least 50 million dollars to the Massachusetts General Hospital; Johnson & Johnson has given 20 million or more to the Scripps Clinic and Research Foundation; Du Pont, about 5 million to Harvard University; Monsanto has pledged 23.5 million to Washington University, St. Louis; Exxon, 7 million to the Cold Spring Harbor Laboratory (Newsday, 1982); and MIT will receive 6-8.5 million from W.R. Grace (ICN, 1983). Additional information on institutional arrangements is contained in a recent publication of the OTA (Commercial..., 1984). And in 1985, acquisitions began to occur: for example, Hybritech by Eli Lilly and Genetic Systems by Bristol-Myers for 300 and 244 million dollars, respectively (C&EN, Bristol-Myers..., 1985).

Likely Timing of Commercial Introduction

There is an intense competition among the hundreds of genetic engineering companies to commercialize their products as rapidly as possible. They all wish to gain initial marketing advantages and to convert their balance sheets into the black after several years of deficit spending on research and development. All of the products in tables 1-4 and powerful DNA probes should be introduced during the 1980s. Biosynthetic insulin (Humulin) was approved by the FDA in 1982, and biosynthetic hGH (Protropin) in 1985 (C&EN, Biosynthetic..., 1985). Contrary to what one would expect, approval and control of bovine interferon is slated to reside with the FDA, not the USDA (GEN,v3/6, 1983). Many lymphokines and cytokines are in clinical trials now. Monoclonal antibody for calf scours and a cloned vaccine for pig scours have both been approved for general use in the United States by the USDA. Large quantities of the single-cell protein Pruteen are now available. Embryo transfer is in extensive use on a ranch (Rio Vista Farms) in Texas (King, 1982), and has been commercialized by GEI in Colorado. It is being offered by a few veterinarians to farmers in other areas of the country. Gene insertion into livestock has occurred, and sex determination of embryos using monoclonal antibodies will soon be routine. The cloned vaccine for foot-and-mouth disease should be in field trials by the mid-1980s, and several other of the cloned animal vaccines should also be approved during the 1980s.

Potential Primary Impacts

Genetic engineering promises to provide substantial benefits to the health and productivity of livestock and poultry. Monoclonal antibodies and DNA probes will markedly improve diagnostic and treatment procedures. The cloned and other genetically engineered vaccines under development should provide greater control of animal diseases than heretofore possible, particularly highly intractable ones such as scours, foot-and-mouth disease, brucellosis, bovine leukosis, pseudorabies, bovine rhinotracheitis, Rift valley fever (C&EN, Agricultural Biotech..., 1985) and bluetongue. Genetically engineered products for scours are already available to the U.S. farmer: a monoclonal antibody for use in calves and a cloned vaccine for swine. The cloned animal growth hormones will promote faster and increased growth in livestock and poultry, when practical methods of administration are developed or when the cloned hormone genes have been transferred permanently into the germplasm. Similar insertion of genes for various cloned lymphokines should increase the resistance of animals to diseases. Intranasal application of interferon already shows promise in protecting calves against bovine rhinotracheitis. Embryo transfer will soon upgrade the quality of livestock, especially of cattle; and in combination with monoclonal antibody technology, it is permitting embryo sexing. Recombinant DNA technology should reduce the cost of single-cell protein, making it competitive with fertilizer- and labor-intensive crops that, moreover, are rainfall dependent and cause extensive soil erosion.

References

1. Audibert, F., Jolivet, M., Chedid, L., et al., "Successful Immunization With a Totally Synthetic Diptheria Vaccine," Proc. Natl. Acad. Sci. USA 79:5042-5046, 1982.

2. Bachrach, H.L., "Recombinant DNA Technology for the Preparation of Subunit Vaccines," J. Amer. Vet. Med. Assoc. 181:992-999, 1982.

3. Bachrach, H.L., "Molecular Approaches to Vaccines," Proc. Intl. Conf. on Genetic Engineering of Animals (New York: Plenum Press, in press).

4. Bachrach, H.L., Callis, J.J., Brown, F., et al., "Achievements in Genetic Engineering and their Influence on the Control and Prevention of Animal Diseases," 51st General Session of the O.I.E., 30 pp., Paris, 1983.

5. Baum, R., "Molecular Computers Are Far From Realization," Chemical & Engineering News 3:29, 1983.

6. Brotman, H., "Engineering the Birth of Cattle," The New York Times Magazine, May 15, 1983, p. 106.

7. Chemical & Engineering News (C&EN), "Gene Cloned for Enzyme to Make Cheese," Feb. 15, 1982, p. 34.

8. ibid., "E. Coli Engineered to Digest Cellulose," March 14, 1983, p. 25.

9. ibid., "Single Cell Protein Process Targeted for Licensing," Aug. 1, 1983, p. 21.

10. ibid., "Virus-Made Protein Acts Like Growth Factor," Sept. 26, 1983, p. 18.

11. ibid., "U.S.S.R. May Buy ICI Protein Know-How," Jan. 16, 1984, p. 20.

12. ibid., "Some Genes Turn On In Many Mouse Cancers," Jan. 2, 1984, p. 22.

13. ibid., "Bristol-Myers Agrees to Buy Genetic Systems," Oct. 28, 1985, p. 9.

14. ibid., "Biosynthetic Growth Hormone Sale Approved," Oct. 28, 1985, p. 9.

15. ibid., "Agricultural Biotech Firm Rides Animal Health Products to Market," Oct. 28, 1985, p. 12.

16. ibid., "Second Oncogene Linked to Growth-Factor Protein," Feb. 20, 1984, p. 7.

17. Cohen, G.H., Dietzschold, B., Ponce de Leon, M., et al., "Localization and Synthesis of an Antigenic Determinant of Herpes Simplex Virus Glycoprotein D that Stimulates the Production of Neutralizing Antibody," J. Virol. 49:102-108, 1984.

18. Davis, B.D., "The Two Faces of Genetic Engineering in Man," Science 219:1381, 1983.

19. D'Eustachio, P., "Gene Mapping and Oncogenes," American Scientist 72:32-40, 1984.

20. Dreesman, G.R., Sanchez, Y., Ionescu-Matiu, I., et al., "Antibody to Hepatitis B Surface Antigen After a Single Inoculation of Uncoupled Synthetic HBsAg Peptides," Nature 295:158-160, 1982.

21. Eckhouse, J., "The High-Tech Cows," San Francisco Chronicle, Jan. 13, 1984, p. 1.

22. Ellis, J., Ozaki, L.S., Gwadz, R.W., et al., "Cloning and Expression in E. Coli of the Malarial Sporozoite Surface Antigen Gene from Plasmodium Knowlesi," Nature 302:536-538, 1983.

23. Genetic Engineering News (GEN) vol. 2, Nos. 1-6, 1982; vol. 3, Nos. 1-6, 1983; vol. 4, No. 1, 1984.

24. Glaubiger, C., Nazerian, K., and Velicer, L.F., "Marek's Disease Herpesvirus IV. Molecular Characterization of Marek's Disease Herpesvirus A Antigen," J. Virol. 45:1228-1234, 1983.

25. Industrial Chemical News (ICN), "Cloned Cells Could Check Chicken Coccidiosis," July, 1982, p. 11.

26. ibid., "Technology Briefs," Sept., 1983, p. 12.

27. ibid., "Monoclonal Antibodies Debut but not as Magic Bullets," vol. 5: 6-7, 1984.

28. Hammer, R.E., Pursel, V.G., Rexroad, C.E., Jr., et al., "Production of Transgenic Rabbits, Sheep and Pigs by Microinjection," Nature 315:680-683, 1985.

29. Kennedy, R.C., Adler-Storthz, K., Henkel, R.D., et al., "Immune Response to Hepatitis B Surface Antigen: Enhancement by Prior Injection of Antibodies to the Idiotype," Science 221:853-855, 1983.

30. Kew, O.M., Nottay, B.K., Natch, M.H., et al., "Multiple Genetic Changes Can Occur in the Oral Poliovaccines Upon Replication in Humans," J. Gen. Virol. 56:337-347, 1981.

31. King, W., "Microscopic Techniques Have a Gigantic Effect on Cattle Breeding Industry," The New York Times, Dec. 4, 1982.

32. Kolata, G., "The Magic in Magic Bullets," Science 222:310-312, 1983.

33. Leong, D., Coleman, K.D., and Murphy, J.R., "Cloned Fragment A of Diptheria Toxin is Expressed and Secreted into the Periplasmic Space of Escherichia Coli K12," Science 220:515-517, 1983.

34. Marshall, E., "USDA Seeks More Basic Biological Research," Science 219:822, 1983.

35. McAuliffe, K., and McAuliffe, S., "Keeping Up With The Genetic Revolution," The New York Times Magazine, Nov. 6, 1983, p. 41.

36. McGrath, J., and Solter, D., "Nuclear Transplantation in the Mouse Embryo by Microsurgery and Cell Fusion," Science 220:1300-1302, 1983.

37. McGraw-Hill's Biotechnology Newswatch (BN), June 7, 1982, p. 7.

38. ibid., vol. 3, No. 4:2, 1983.

39. Miller, J.A., "Spelling Out a Cancer Gene," Science News 122:316-319, 1982.

40. Miller, R.A., Maloney, D.G., Warnke, R., et al., "Treatment of B-Cell Lymphoma with Monoclonal Anti-Idiotype Antibody," N. Engl. J. Med. 306:517-522, 1982.

41. Miller, W.L., Coit, D., Baxter, J.D., et al., "Cloning of Bovine Prolactin cDNA and Evolutionary Implications of Its Sequence," DNA 1:37-50, 1981.

42. Newsday (by B.D. Colen), "Academic Marriages of Convenience," July 7, 1982.

43. Palmiter, R.D., Norstedt, G., Gelinas, R.E., et al., "Metallothionein-Human GH Fusion Genes Stimulate Growth of Mice," Science 222:809-814, 1983.

44. Reagan, K.J., Wunner, W.H., Wiktor, T.J., et al., "Anti-idiotype Antibodies Induce Neutralizing Antibodies to Rabies Virus Glycoprotein," J. Virol. 48:660-666, 1983.

45. Roizman, B., Warren, J., Thuning, C.A., et al., "Application of Molecular Genetics to the Design of Live Herpes Simplex Virus Vaccines," Devel. Biol. Standard. 52:287-304, 1982, S. Karger, Basel.

46. Schmeck, H.M., Jr., "Activity of Genes Reported Altered in Treating Man," The New York Times, Dec. 9, 1982, p. A1.

47. Schmeck, H.M., Jr., "Researchers Correct Defect in a Human Gene," The New York Times, Apr. 26, 1982, p. A1.

48. Schmeck, H.M., Jr., "Strange Alliance: Legions of Bacteria Created to Serve Man," The New York Times, Jan. 1, 1984.

49. Science News (SN), "Alternative in Antibody Production," Feb. 19, 1983, p. 125.

50. Seeburg, P.H., Sias, S., Adelman, J., et al., "Efficient Bacterial Expression of Bovine and Porcine Growth Hormones," DNA 2:37-45, 1983.

51. Seidel, G.E., Jr., "Superovulation and Embryo Transfer in Cattle," Science 211:351-358, 1981.

52. Sella, J., "Remarks Before the Los Angeles Soc. of Financial Analysts," Dec. 8, 1983, Amer. Cyanamid Co., Wayne, N.J.

53. Smith, B.P., Reina-Guerra, M., Hoiseth, S.K., et al., "Aromatic-Dependent *Salmonella typhimurium* as Modified Live Vaccines for Calves," 1983.

54. Smith, G.L., Mackett, M., and Moss, B., "Infectious Vaccinia Virus Recombinants that Express Hepatitis B Virus Surface Antigen," Nature 302:490-495, 1983.

55. Smith, G.L., Murphy, B.R., and Moss, B., "Construction and Characterization of an Infectious Vaccinia Virus Recombinant that Expresses the Influenza Hemagglutinin Gene and Induces Resistance to Influenza Virus Infection in Hamsters," Proc. Natl. Acad. Sci. USA 80:7155-7159, 1983.

56. Stewart, A.G., and Bell, L.D., "Production of Relaxin with Recombinant-DNA Technology, European Patent Office 068375, June 1982.

57. Sukumar, S., Pulciani, S., Doniger, J., et al., "A Transforming *ras* Gene in Tumorigenic Guinea Pig Cell Lines Initiated by Diverse Chemical Carcinogens," Science 223:1197-1199, 1984.

58. The New York Times (NYT), "Whether to Make Perfect Humans," July 22, 1982, A22.

59. ibid., "Gene-Splicing Project Using Diptheria Approved," Jan. 1, 1983, p. 20.

60. ibid., "Birth Results From Transferred Embryo," Feb. 4, 1984, p. 6.

61. Time Magazine, "Hoping to Clone Some Profits," Feb. 27, 1984, p. 76.

62. U.S. Congress, Office of Technology Assessment (OTA), Impacts of Applied Genetics: Micro-Organisms, Plants, and Animals, Washington, DC, 1981.

63. ibid., Commercial Biotechnology, An International Analysis, OTA-BA-218, Washington, DC, Jan. 1984.

64. U.S. Department of Agriculture, Notes From The Director, No. 148, ARS, NER, BARC, "Genetic Engineering Agreements Signed," July 1983, Beltsville, MD.

65. Winter, A.J., Verstreate, D.R., Hall, C.E., et al., "Immune Response to Porin in Cattle Immunized with Whole Cell, Outer Membrane and Outer Membrane Protein Antigens of *Brucella abortus* Combined with Trehalose Dimycolate and Muramyl Dipeptide Adjuvants," Infect. Immun. 42:1159-1167, 1983.

66. Wood, T.G., McGeady, M.L., Blair, D.G., and Vande Woude, G.F., "Long Terminal Repeat Enhancement of v-*mos* Transforming Activity: Identification of Essential Regions," J. Virol. 46:726-736, 1983.

Table 1. Genetically engineered pharmaceuticals and nutrients.[1]

Nutrients/Antibiotics

amino acids

- proline
- L-phenylalanine
- serine[2]
- threonine[2]
- tryptophane[2]
- aspartic acid[2]

single-cell proteins

- Pruteen (ICI)
- Provesteen (Phillips)
- Probion (Hoechst)

antibiotics

- enhancements

Hormones

- somatostatin
- insulin
- human growth hormone, hGH
- bovine " " , bGH
- porcine " " , pGH
- chicken " " , cGH
- hGH releasing factor[3]
- human chorionic gonadotropin, hCG
- bovine prolactin[4]
- porcine prolaxin
- beta endorphin
- calcitonin
- thymosin alpha
- erythropoietin

Enzymes/Proteins

- urokinase
- tissue plasminogen activator
- clotting factor VIII[4]
- rennin
- cellulase
- subtilisn
- reverse transcriptase
- luciferase
- beta lactomase
- human serum albumin
- diphtheria toxin
- alpha-1 antitrypsin

---

[1] Most are cloned; s-c proteins and antibiotics are enhancements in microorganisms. [2] Under development. [3] And chemically synthesized. [4] Gene cloned but not expressed.

Table 2. Some bioengineered subunit protein vaccines under development.

Viral

bovine and human warts
adeno
Marek's, bovine rhinotracheitis, pseudorabies, herpes, equine herpes
feline panleukopenia, canine and porcine parvo, Aleutian mink disease
bluetongue, calf rota
foot-and-mouth disease, polio, hepatitis A
swine transmissible gastroenteritis
influenza, fowl plague
Rift valley fever
rabies, vesicular stomatitis
hepatitis B
bovine leukosis

Bacterial

enterotoxigenic *E. coli* (pig and calf scours)
gonococcus
syphilis
dental caries
pertussis
meningitis (not proteins)

Parasitic

avian coccidiosis
malaria

Table 3. Several uses of monoclonal antibodies (Mab).

1. Purification of antigens on Mab-affinity columns.
2. Diagnosis, passive immunization (e.g.,calf scours) and treatment of infectious diseases.
3. Testing of meats and foods for bacterial contaminations.
4. Treatment of autoimmune diseases using anti-idiotype Mab.
5. Anti-idiotype Mab as vaccines.
6. Mab as antitoxins and antivenoms.
7. Preventing the rejection of organ transplants.
8. Monitoring levels of hormones and of therapeutic and illegal drugs.
9. Pregnancy tests using Mab to human chorionic gonadotropin.
10. Post-coital reversible contraception (Mab blocking of progesterone).
11. Sexing of livestock embryos.
12. Identifying antigenic, immunogenic, pathogenic and tumor sites on proteins, infectious agents and cells.
13. Monitoring tumor-indicative antigens (carcinoembryonic antigen).
14. Imaging tumors with radioisotopically-labeled Mab.
15. Targeting and destroying cancer cells with Mab conjugated to chemicals, toxins, venoms or enzymes.
16. Inducing remission of B-cell lymphomas with patient-specific Mab.

Table 4. Genetically engineered lymphokines and cytokines.

1. alpha, beta, gamma and hybrid classes of human interferons.
2. alpha, beta and gamma bovine interferons.
3. consensus human interferon incorporating features of the 13 alpha interferon subtypes.
4. human interleukin-2 (IL-2)
5. colony stimulating factor (CSF).
6. tumor necrosis factor (TNF).

Table 5. Improvements in animals from embryo manipulations.

Embryo transfers

- sex pre-selection
- twinning
- compression of generation times
- shipping embryos rather than animals for breed improvement programs
- extending past death the reproductive lives of donors
- stopping biological time for centuries
- rapid upgrading of herd quality
- increases in food and fiber production
- increased resistance to diseases
- saving of endangered species

Anticipated from insertions of cloned genes

- fertility gains
- multiple birthings
- eased birthings
- improved digestion
- more efficient feed utilization
- utilization of lower grade feeds
- more rapid growth
- improved size and conformation
- improved quality and quantiy of food and fiber production
- increased disease resistance
- engineering of all purpose animals
- tailoring of animal hardiness for different environments
- possible revival of extinct species (nuclei replacements)

Fig. 1. Synthesis of proteins by transcription and translation in animal cells. The DNA genes in the nucleus contain recipes for manufacturing thousands of different proteins. However, the proteins are not made directly from the genes. The recipes are first transcribed individually into messenger RNA molecules (mRNAs) which then migrate to cytoplasmic biomachines called ribosomes for translation into protein molecules.

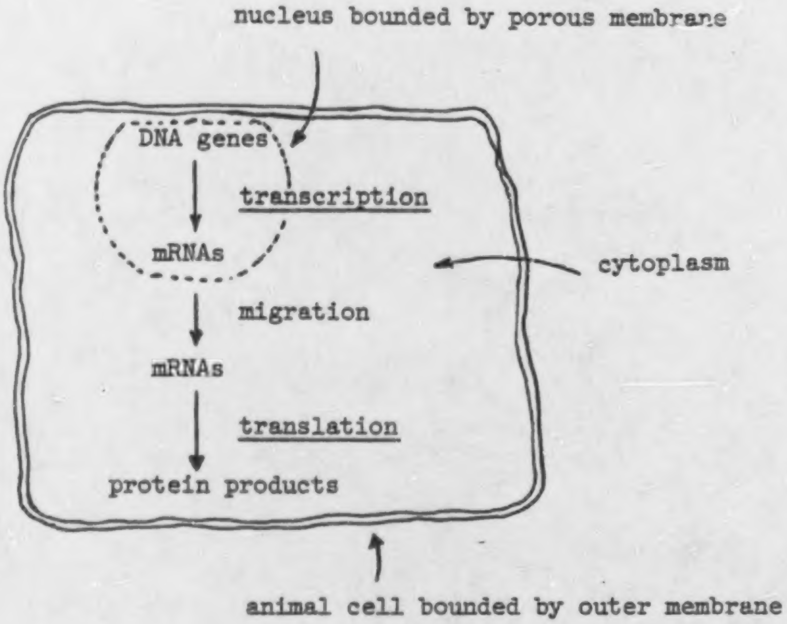
Fig. 2. The genetic code. Each of the 64 combinations of the 4 letters (U, C, A, G) of mRNAs taken 3 at a time, called codons, directs the insertion of a specific amino acid into a growing protein chain or terminates it (0). Amino acid abbrev: phe,phenylalanine; leu,leucine; ile,isoleucine; met,methionine; val,valine; ser,serine; pro,proline; thr,threonine; ala,alanine; tyr,tyrosine; his,histidine; gln,glutamine; asn,asparagine; lys,lysine; asp,aspartic acid; glu,glutamic acid; cys,cysteine; trp,tryptophan; arg,arginine; gly,glycine.

Fig. 3. A. Recombinant DNA procedure. An animal gene is spliced into a carrier DNA (called a vector) for insertion into a microorganism (a bacterium is shown) or alternate animal host cell, and is made to replicate and express its protein product. The vector shown is a bacterial plasmid, but it can also be the double-stranded DNA of a virus.

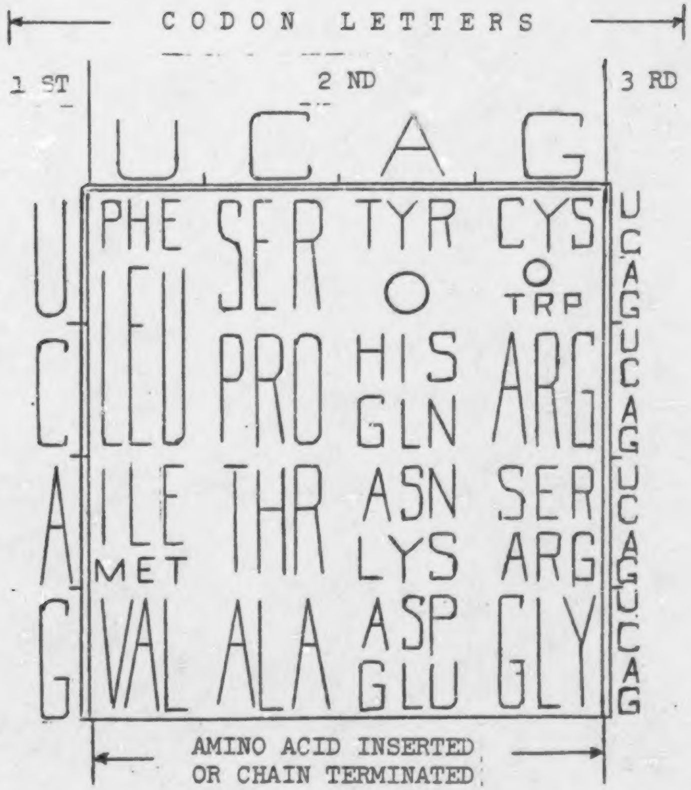
B. Monoclonal antibody production. Antibody-producing spleen cells from an immunized mouse are fused with well-characterized mouse myeloma cells. The fused cells, called hybridomas, have the perpetual growth characteristics of the myeloma cells and the spleen cell's ability to produce multiple copies of a single species of antibody molecule reactive with a specific site on the antigen used to immunize the animal. Different hybridomas in the mixture are grown separately, giving rise to pure clones of hybridomas that produce a single species of monoclonal antibody.

Figure Captions cont'd

c. Embryo transfer. Artificial transfers of embryos from a donor animal to surrogate mothers. Before transfer, the isolated embryos can be treated in a variety of ways -- including splittings, fusion with embryos of other animal species, injection of pronuclei of ovum embryos with cloned foreign genes (this gene cloning in mammals has been done to date in mice, rabbits, sheep, and pigs, see text) and freezing for storage.



nucleus bounded by porous membrane
DNA genes
transcription
mRNAs
cytoplasm
migration
mRNAs
translation
protein products
animal cell bounded by outer membrane

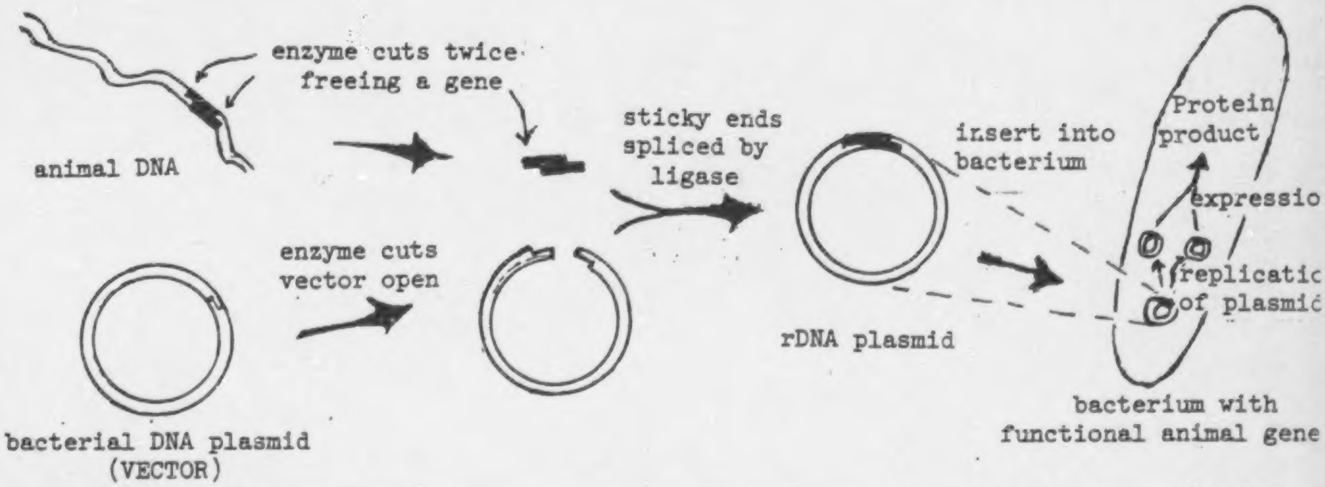


CODON LETTERS

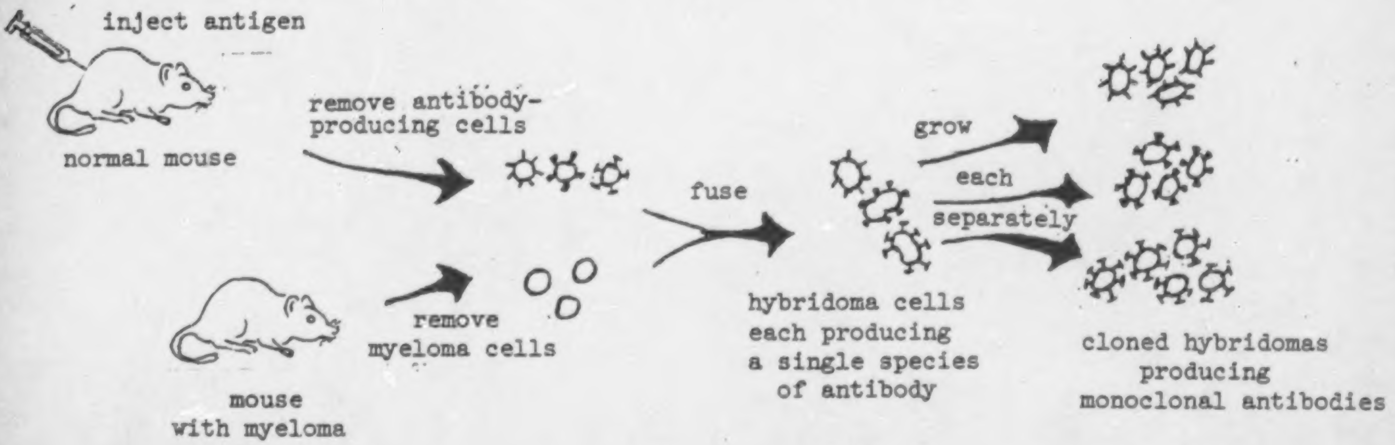
1 ST	2 ND U	2 ND C	2 ND A	2 ND G	3 RD
U	PHE LEU	SER	TYR O	CYS O TRP	U C A G
C	LEU	PRO	HIS GLN	ARG	U C A G
A	ILE MET	THR	ASN LYS	SER ARG	U C A G
G	VAL	ALA	ASP GLU	GLY	U C A G

AMINO ACID INSERTED
OR CHAIN TERMINATED

A. RECOMBINANT DNA PROCEDURE

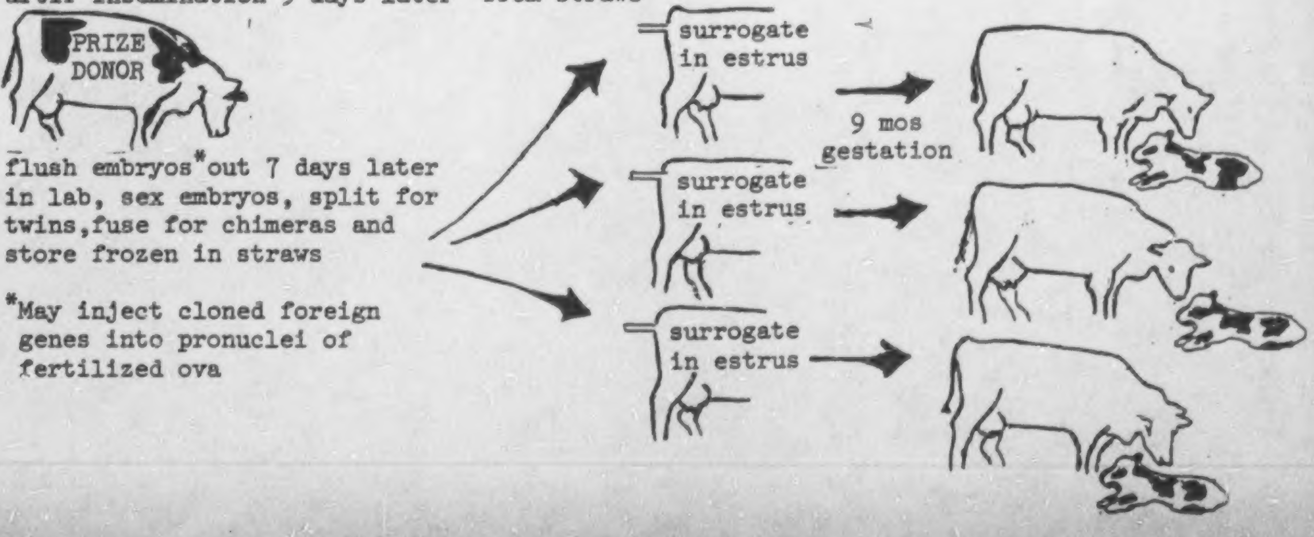


B. MONOCLONAL ANTIBODY PRODUCTION



C. EMBRYO TRANSFER

1. superovulate with hormones
2. artif insemination 5 days later
5. nonsurgical
embryo transfers
from straws



3. flush embryos* out 7 days later
4. in lab, sex embryos, split for twins, fuse for chimeras and store frozen in straws

*May inject cloned foreign genes into pronuclei of fertilized ova

Background Paper No. 2

Animal Reproduction

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U.S. Congress

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ANIMAL REPRODUCTION

Summary

The field of animal reproduction is in the midst of a scientific revolution that could scarcely have been visualized a decade ago. Indeed, if all of the technology now available were used, a new kind of animal breeding system could be put into operation within 10 years.

By the year 2000 artificial insemination may be replaced by a system best characterized as "artificial embryonation." In this system highly trained technicians will place embryos into the uteri of groups of outstanding female animals whose estrous cycles have been regulated by artificial means; for example, by administering hormones through injections, ear implants, or intravaginal devices. The ova from this "superovulation" will be collected surgically or non-surgically (by flushing) and then fertilized in the laboratory by spermatozoa from outstanding males. The fertilized ova can then be cultured, frozen, and stored until needed. Finally, the embryos will be placed in foster mothers non-surgically.

Ultimately, it may be possible to sex the embryos by separating the X-bearing and Y-bearing spermatozoa or by identifying the male embryos by immunological techniques so that recipient beef cows will receive primarily male embryos and dairy cows will receive primarily females. Techniques for reducing early embryonic deaths, the major cause of infertility in all farm animals, are also likely to be developed within this time frame.

Achieving these goals will entail funding research in three major areas: (1) the development of improved estrous cycle regulation techniques, (2) the development of improved techniques for

superovulation, embryo collection, storage, sexing, and transfer, and (3) the development of methods for reducing embryo mortality and improving fertility in all classes of farm animals.

Vigorous pursuit of research in these areas could result, by the year 2000, in the marketing of genetically engineered embryos containing genes that will improve fertility and fecundity and result in improved rates of gain, improved carcass characteristics, increased milk production, and increased resistance to diseases in offspring. Despite recent spectacular breakthroughs in introducing human genes into laboratory animals, a great deal remains to be learned about the factors that control chromosomal integration of foreign DNA, its retention during embryonic development, and ultimately its expression, without disruption of embryogenesis. These developments will affect the major drug companies, genetic engineering companies, equipment manufacturers, veterinarians, inseminators, and extension workers, as well as the Nation's farmers.

The ultimate goal of this research is to increase the efficiency of production so that fewer animals can produce the needed animal products with less labor input.

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Figure 3. Schematic presentation of cow embryo transfer procedures. (From G. E. Seidel, Jr., Science 211: 351, 1981; Copyright 1981 by The American Association for the Advancement of Science).

I. INTRODUCTION

A. Definition and Scope

The field of Reproductive Biology contributes directly to solution of the two major problems in the world today. On the one hand, those of us who work in this field are able to use our findings to develop new and improved contraceptive methods for controlling the world's population growth and, on the other hand we are often able to use the same data to devise methods for improving the efficiency of reproduction in our domestic animals, resulting in more efficient production of animal products.

In order to fully appreciate the impacts of advances to be expected in Animal Reproduction during the next twenty years, it is essential to understand that food produced from livestock and poultry represents more than half of that consumed in the United States. Animal products supply two-thirds of the proteins, one-third of the energy, one-half of the fat, four-fifths of the calcium and two-thirds of the phosphorous, as well as significant quantities of essential minerals, micronutrients and vitamins in our daily diets. The world's population continues to increase, and it has been estimated that increased world population and increased consumer buying power will increase the world requirements for food energy from animal sources from 574 to 953 billion Mcal between 1970 and 2000; 70% of this increased demand will be supplied by ruminants, 19% by pigs and 11% by poultry. Similarly, the world requirement for food protein from animal sources will increase from 22 to 38 million metric tons during this period and it is estimated that ruminants will supply 75%, pigs 6% and poultry 19% of the increased demand (Fitzhugh et al., 1978). This increased demand can best be met by increasing the productivity of individual animals by improving fertility, health, nutrition and genetic potential. It is in this category - increased productivity per animal - that we are likely to see our greatest progress during the next 20 years. Ruminant animals, by virtue of their ability to obtain nutrients from grass and other forages that man cannot directly utilize have always been an important source of food. Indeed, it appears that the ruminant animals will be the ones that will continue to contribute the most to food energy and protein requirements of the world, and they will continue to make up a large percentage of our gross national product (Hansel, 1984).

Those of us who work in the field of Animal Reproduction find ourselves in the midst of a scientific revolution that could scarcely have been visualized 10 years ago. We have now acquired the capability to apply our knowledge of the hormonal control of ovarian functions in farm animals to synchronize the estrous cycles of groups of animals; to produce superovulation in cattle, sheep and swine; to collect, by both surgical and non-surgical methods, the large numbers of ova shed; to fertilize these ova in vitro or in vivo; to store the resultant embryos frozen for indefinite periods of time and, ultimately transfer them to foster mothers by non-surgical techniques. Indeed, we may have now reached the point where we may visualize, by the year 2000, the development of an animal breeding system in which artificial insemination may be replaced by a system best characterized as "artificial inembryonation". In this system highly trained technicians will place embryos into the uteri of groups of cows, ewes, or sows whose estrous cycles have been previously regulated by artificial means so that all will be in the proper stage. It is likely that the ova, from which these embryos have been produced will have been collected from outstanding females, fertilized in the laboratory by spermatozoa from outstanding males, cultured and stored frozen until needed. Ultimately, it may be possible to sex the embryos, either by use of techniques for separating the X- and Y- bearing spermatozoa or by identifying the male embryos by immunological techniques, so that recipient beef cows will receive male embryos and dairy cows will receive females. Techniques for reducing early embryonic deaths, the major causes of infertility in all farm animals, are also likely to be developed within this time frame.

B. Major Lines of Research to be Pursued:

In order to achieve these developments four lines of research are likely to be vigorously pursued. These include:

(1) The development of improved estrous cycle regulation techniques to enable either insemination or "inembryonation" to be carried out at pre-set times.

(2) The development of improved techniques for superovulation, embryo transfer and other embryo manipulations.

(3) The development of methods for improving fertility.

(4) The development of methods to alter productive traits in domestic animals by genetic engineering techniques, including the introduction of new genes into embryos.

An attempt will be made to assess the current state of the art in each of these areas and to assess developments that may be expected by the year 2000, provided research is funded at an adequate level.

II. RESEARCH TRENDS

A. Cycle Regulation

1. State of the Art

Hansel and Milvae (1984) recently summarized the current status of developments in regulation of ovulation in cattle, sheep and swine. The early attempts at estrous cycle regulation were based on the use of progestational compounds, such as 6 methyl-17-acetoxy progesterone (MAP), chlormadinone acetate (CAP), melengestrol acetate (MGA), fluorogestone acetate (FGA) and 17α-acetoxy-11β-methyl,19-nor-preg-4-ene-20-dione (Norgestomet). These compounds,administered orally or as implants, resulted in synchronization of estrus over a 4 day period beginning approximately 48 hours after withdrawal of the progestational compound. However, conception rates of cattle bred at the synchronized estrus were generally about 15% lower than those for control animals bred at a normal estrous cycle.

The next phase of cycle synchronization in cattle began about 1972 following discovery of the luteolytic effects of prostaglandin $F_2\alpha$ ($PGF_2\alpha$) (See Hansel and Convey, 1983, for review). It was found that exogenous $PGF_2\alpha$ was luteolytic - that is, it caused regression of the corpus luteum when given between days 5 and 16 of the estrous cycle and that estrus and ovulation followed administration of a luteolytic dose by 3 to 4 days. Administration of $PGF_2\alpha$ during the first 5 days of the cycle was ineffective. Several treatment regimens for using $PGF_2\alpha$ for cycle regulation were developed. These included: (1) a regimen in which two doses of $PGF_2\alpha$ were given 11 days apart and the animals were then inseminated at the estrus following the second injection and (2) a regimen in which animals were bred as they came into a naturally occurring estrus during a 5-day period; the remaining cattle were then treated with $PGF_2\alpha$ on the 5th day of the treatment period and bred at the ensuing estrus. Both regimens gave encouraging results but conception rates of animals synchronized by the first method were lower than for controls. The second method suffered the disadvantage that about one-fourth of the cattle in any group had to be bred at an unsynchronized estrous period.

Recently, methods have been developed that depend upon the combined use of progesterone (or progestational compounds), and

$PGF_2\alpha$ treatments. Progestational compounds are administered either by a progesterone releasing intravaginal device (PRID) or a progestagen-containing ear implant for 7 days, in conjunction with $PGF_2\alpha$ injected on the 6th day. These combined PRID-$PGF_2\alpha$ treatments appear to be the most effective, because of their simplicity and because the improved synchronization of estrus they provide allows insemination at pre-set times. Thus, it is not necessary to check animals for estrus, a time consuming and expensive operation. Conception rates equal to those for control animals have been obtained by these treatments. Another method currently in use consists of insertion of a synthetic progestagen (Norgestomet) as an ear implant which remains in place for 9 days. An estrogenic hormone, estradiol valerate, is injected subcutaneously at the same time the Norgestomet is implanted into the ear. Animals are bred between 48 and 54 h after implant removal.

Recent studies indicate that the PRID-$PGF_2\alpha$ method for estrous cycle synchronization is effective in inducing, as well as synchronizing estrus in beef cattle, resulting in induction of fertile estrus in a significant number of anestrous cows. Figure 1 illustrates the extent to which this treatment shortens the breeding season in beef cattle. This is an important result, not only because of the improved efficiency that results in the breeding operations but because it results in early calving. Beef calves born early in the calving season usually grow faster and make more efficient gains than those that are born later in the calving season.

Intravaginally administered progestagens (MAP,FGA) are also effective in controlling estrus and ovulation in ewes during the breeding season (See Hansel and Convey, 1983). The addition of a gonadotropic hormone, usually pregnant mare serum (PMSG), to the progestagen treatment results in a tighter sychrony of estrus and allows ewes in late anestrus to be synchronized. Recently, a 7-day combined progestagen-$PGF_2\alpha$ treatment has been found effective in ewes, as it is in cows. Excellent synchronization and conception rates resulted from administration of MAP as a vaginal pessary for 7 days combined with single injections of $PGF_2\alpha$ on the 6th day of the progestagen treatment.

The most promising method for controlling estrus and ovulation in the sow is by use of the orally active synthetic progestagen, allyl trenbolone, (17α-allyl-estratriene 4-9-11, 17βol-3-one;AT). Oral doses of 12.5 mg, or more of AT per day dependably result in effective control and synchronization of estrus, without causing a reduction in fertility or adverse side effects. Unfortunately, $PGF_2\alpha$ has not proved useful in cycle regulation in pigs because it does not regress the corpus luteum, unless administered after day 12 of the estrous cycle.

2. Future Developments

The next generation of estrous cycle control techniques will probably involve the use of simple methods for extending the functional lifespan of the corpus luteum by 6 or more days, thereby overcoming the problem of the ineffectiveness of $PGF_2\alpha$ when administered during the first 5 days of the estrous cycle. Ideally, such a method would be based on a single injection of a potent luteotropic substance, followed 6 days later by administration of a single luteolytic dose of $PGF_2\alpha$, or a potent analog, and followed by a single insemination 80 hours later. This method would eliminate the need to administer progestational compounds by implants or PRIDs and would thus require less handling of the animals, an important consideration in beef cattle operations.

A development of this sort depends upon finding one or more potent luteotropic compounds that may be used to extend the functional lifespan of the corpus luteum. Several methods show promise for achieving this end. One method involves manipulation of the metabolites of arachidonic acid and other prostaglandins. It has recently been found that prostacyclin (PGI_2), a metabolite of arachidonic acid, produced by the cyclooxygenase pathway is capable of stimulating progesterone production by the corpus luteum and prolonging its lifespan. It also appears that products of the lipoxygenase pathway of arachidonic acid metabolism, such as 5 hydroxyeicosatetraenoic acid (5-HETE), inhibit prostacyclin synthesis and have a luteolytic effect in the normal animal. Thus, it may be possible to develop long-acting potent analogs of PGI_2 that will prolong the functional life of the corpus, or to develop compounds that block the production of 5-HETE by the lipoxgenase pathway, to achieve the same effect. It seems now clear that the prostaglandins play many important roles in ovarian functions and research on this class of compounds will be continued.

A second method of extending the functional lifespan of the corpus luteum of non-pregnant animals is by use of potent gonadtropin releasing hormone analogs (GnRH). Repetitive treatments with a potent GnRH analog will increase the secretion of LH from the pituitary, which, in turn, elevates plasma progesterone concentrations and extends the functional lifespan of the corpus luteum 6-8 days. These new methods will not only simplify estrous cycle regulation, they will also be safer, and will probably improve the conception rates of the treated animals.

B. Superovulation, Embryo Transfer and Embryo Manipulations

1. State of the Art

A general understanding of the mechanisms controlling follicle growth, ovum maturation and ovulation is required in order to understand the current superovulation schemes and their limitations. As may be seen from Figure 2, the two anterior pituitary hormones, follicle stimulating hormone (FSH) and luteinizing hormone (LH), play the major roles in follicle growth, ovum maturation and ovulation. The surge release of LH from the pituitary near the onset of estrus results in ovulation.

FSH plays a role in recruitment of pre-antral follicles and in inducing estrogen synthesis by the granulosa cells in the follicle. Estrogen, produced by these follicles, as well as progesterone from the corpus luteum, feed back negatively on pituitary LH secretion. However, following corpus luteum regression and a slight increase in LH secretion, follicular maturation occurs and a sharp rise in estrogen follows. At this point, estrogen exerts a positive feedback effect on LH release and the LH surge and ovulation occur in rapid sequence. LH then becomes the major luteotropic hormone responsible for maintenance of the corpus luteum and for the secretion of its major hormone, progesterone during a large part of the estrous cycle.

Understandably then, exogenous FSH and LH, or substitutes for them, are the major tools used in producing superovulation. For example, the treatment of prepubertal gilts with 750 IU of pregnant mare serum (PMSG), a substitute for FSH, followed by 500 IU of human chorionic gonadotropin (HCG), a substitute for LH, synchronizes the production of a large, uniform crop of ovarian follicles that ovulate 42 h after the HCG treatment. Similarly, injection of PMSG (400-600 IU) into either cycling or anestrous ewes previously treated for 12 days with a progesterone-like compound (FGA) administered intravaginally, results in ovulation of increased numbers of fertile ova. This treatment has been used to maintain fertility during the entire year in ewes, which are normally seasonal breeders.

Techniques for superovulation in cattle usually consist of injections of FSH, or mixtures of FSH and LH, for 3 days, beginning about day 10 of the normal estrous cycle, in order to initiate follicle growth. These injections, are followed by one or more injections of prostaglandin $F_2\alpha$ ($PGF_2\alpha$) to cause regression of existing corpora lutea. The animals may then be given one or more injections of LH to insure that ovulation occurs. However, LH treatment may not be necessary since most animals are capable of releasing adequate amounts of endogenous LH under these conditions. A schedule of injections representative of that used by many large embryo transfer companies is shown in Table 1. Animals are usually inseminated two or more times at the estrus which occurs after the $PGF_2\alpha$ injections. Embryos are then collected non-surgically 7 days after estrus and are either frozen for future use, or transferred non-surgically to synchronized recipients. Superovulation techniques are still imperfect; in cattle, most methods produce an average of less than 6 fertilized ova per treatment. The entire process of superovulation, embryo collection and transfer, as summarized by Seidel (1981) is shown schematically in Figure 3.

A number of manipulations of ova and embryos that may eventually have profound effects on the animal industries can now be carried out. These include non-surgical collection of ova or embryos and nonsurgical transfer to recipient animals, in vitro fertilization, freezing and storage of frozen embryos; sexing embryos, and dividing embryos into two or more parts each capable of developing into a new individual.

Non-surgical embryo recovery and transfer techniques are successful and are now widely used. It is estimated that as many as 100,000 calves may be born in the United States and Canada in 1985 as a result of non-surgical embryo transfers. The procedure involves flushing embryos, or ova, out of the reproductive tract through a cannula inserted into the uterus by way of the cervix, as shown in Figure 3. The estrous cycles of the recipient animals are synchronized with those of the donor animals. The embryos are then transferred to the uteri of the recipient animals in much the same manner as semen is deposited in artificial insemination techniques. Pregnancy rates obtained with non-surgical transfers range from 30% to 60% and, although these rates are lower than those obtained after artificial insemination, there are no indications of increased abortions, stillbirths, abnormal calves or alterations in the sex ratio of the calves born.

Fertilization of ova in vitro has been achieved for mice, rats, rabbits, humans and cattle and pregnancies resulting from transfers of bovine embryos fertilized in vitro have recently been reported. However, only a few successful cases have been reported and a great deal of work remains to be done to perfect in vitro fertilization techniques for cattle, sheep and swine.

Methods for freezing and long-term storage of early bovine embryos, similar to those developed for freezing and storing of bull semen, have recently been developed. These methods depend upon the use of cryoprotectants - usually dimethylsulfoxide or glycerol. Storage is usually at -196°C in liquid nitrogen and about one-third of the embryos are killed or damaged by the freezing and thawing processes. However, pregnancy rates following transfer of the remaining two-thirds of the embryos are similar to those obtained when fresh embryos are transferred. The use of frozen embryos in commercial embryo transfer operations is increasing.

There are at least two methods for determining the sex of bovine and ovine embryos. One method is to detect, by immunological methods, a known product of the Y-chromosome - the HY antigen. This method shows great promise and may soon be ready for commercial application. A second method is to karyotype the embryos. The bovine X and Y chromosomes are easily distinguished when metaphase chromosomes are available. Cells for karyotyping are obtained by removal of the tip of the elongated blastocyst. Some of the embryos are destroyed in this process. In general, when these techniques are perfected, we would expect that female embryos would be transferred into dairy cows, since female calves are more valuable in the dairy industry, while male embryos would be transferred to beef cows, since males (and steers) usually grow faster than females.

Insofar as we are aware, successful methods for separating the X- and Y- bearing bovine spermatozoa are not yet available, although a number of claims have been made in recent years.

The blastomeres of early embryos (2-4 cells) can be separated by microsurgical techniques and sets of identical twins, and even quadruplets, have been produced in this way. Each blastomere is totipotent; that is, it has the capability of developing into an entire individual able to reproduce itself. In a recent further development, it has been possible to dissect the inner cell mass of 7-day bovine blastocysts by microsurgical techniques, enclose each half in a surrogate zona pellucida and obtain live calves from each portion. This surprising development appears to make it possible to further increase the number of offspring obtained from each donor animal.

Chimeras have been produced by many workers by aggregating cleavage stage embryos in vitro. These chimeras, which are ordinarily derived from four parents, are formed by removal of the zona pellucida of 4 or 8 - cell embryos by use of a proteolytic enzyme (pronase), followed by co-culture in a medium containing phytohemagglutinin for about 24 hours. The aggregated embryos are later transferred to other females for gestation. In a most remarkable demonstration of this technique, blastomeres from 8-cell sheep embryos were separated and placed, one at a time, with blastomeres from a 4-cell embryo in surrogate zona pellucidae. Although the resulting embryos were chimeric, the fetus and resulting lambs were derived from the 8-cell embryo and the placentae from the 4-cell embryo. Identical quintuplet sheep have been produced by this procedure (See Seidel, 1983, for review).

2. Future Developments

Superovulation represents the "Achilles heel"of the embryo transfer industry. The numbers of viable ova shed after use of existing superovulation treatments are quite variable and, on the average less than 6 good embryos are produced as a result of each superovulation procedure. The potential for harvesting increased germ plasma by superovulation is illustrated by the fact that bovine ovaries probably contain in excess of 50,000 viable ova at the time of puberty and less than 100 of these are ovulated during the lifetime of an average animal.

We expect that major advances in improving superovulation techniques will occur and will be dependent upon the isolation, identification, and exploitation of a number of presently unidentified ovarian compounds that play local coordinatory roles in the control of ovulation. Several small peptides (less than 2,000 daltons) capable of inhibiting the binding of the gonadotropins to their receptor proteins have been found in both the ovaries and testes. These binding inhibitors may well contribute to the variable results obtained to date with all superovulatory treatment regimens. In addition to these peptides, a substance called inhibin, which acts to selectively inhibit FSH, but not LH, secretion by the anterior pitutitary has been extracted from follicular fluids of sows, mares and cows. Ovarian concentrations of this substance, which also appears to be a small peptide with a molecular weight of less than 19,000 daltons, could greatly influence the results obtained with superovulation treatment regimens. Indeed, it is already known that immunizing ewes against inhibin greatly increases the number of eggs shed at ovulation.

In addition, hypothalamic peptides, including both GnRH-like substances and oxytocin, have been found in relatively large amounts in ovarian tissues and these peptides may play local coordinatory roles in follicle maturation and ovulation, as well as in corpus luteum formation and function (Hansel and Convey 1983). Although the roles of these substances in ovarian function are not fully understood, they are clearly candidates for use in developing improved superovulation techniques. The control of follicle growth and follicular atresia is not well understood, but it is expected that great progress will be made in understanding these processes during the next decade. One can also confidently expect new developments in other aspects of embryo transfer, including perfection of in vitro fertilization techniques for farm animals and improvement of existing techniques for embryo freezing.

There is great potential for controlling the sex of calves, lambs and piglets and further advances in this area may be expected. From time to time, methods have been reported for separating the X- and Y- bearing spermatozoa, but none of these methods have proved practical. It is likely that sperm separation will be achieved before the year 2000. The development of techniques for producing highly specific monoclonal antibodies should prove very useful in this effort. If the X- and Y- bearing sperm contain specific cell surface antigens, it should be possible to develop monoclonal antibodies to them and to use these antibodies as a basis for separating the male and the female producing sperm.

C. Improvement of Fertility

1. State of the Art

Opportunities exist for economic gains as a result of improving the fertility of our farm animals. Less than 60% of mature cows and 65% of heifers produce a calf as a result of one insemination. These large losses are due to fertilization failures, embryonic deaths not attributable to any specific disease, and to reproductive diseases of various kinds. Among these causes, early embryo death is the most important. Thus, research aimed at reducing embryo mortality and improving the fertilization rate offer the best approaches to solution of this problem.

Many attempts have been made to manipulate the hormonal profiles of cattle, sheep and swine during early pregnancy to improve fertility. Exogenous progesterone, given to early pregnant cattle for the purpose of improving reproductive performance, has met with only limited success. However, Shemesh et. al., (1983) recently reported that intravaginal insertion of sponges impregnated with 250 mg of MPA during days 6 - 17 after insemination increased fertility in a group of low-fertility Holstein cows. Several hormonal treatments, notably combinations of progesterone and estrogen, have been studied in attempts to reduce embryonic mortality in swine but success has been limited.

Prolonged postpartum anestrus is another major cause of reduced fertility in cattle and sheep. The causes of prolonged postpartum anestrus are not well understood, but lactation, suckling and inadequate nutrition all contribute to the problem. In addition to induction of estrus by progesterone impregnated intravaginal devices, (PRIDs), as previously discussed, a reduction in the length of the postpartum period has been achieved in dairy cattle by administration of the synthetic progestational compound, melengestrol acetate (MGA) for 14 days, beginning 3 weeks postpartum (See Hansel and Milvae, 1984, for review). The gonadotropin releasing hormone (GnRH), a hypothalamic compound that controls the secretion of LH from the pituitary, has also been used in early postpartum cows to stimulate ovarian activity. Dairy cattle given GnRH at 14 days postpartum had regular estrous cycles and fewer ovarian dysfunctions following the onset of cyclicity.

Cystic ovarian conditions represent a major cause of infertility in cattle; estimates of the incidence of this disease in dairy cattle range from 5.6 to 18.8%. For the most part, these conditions are thought to be due to either an inadequate, or an inappropriately timed, release of LH from the pituitary at the time of estrus. This results in a failure of ovulation, and cystic follicles develop. Both LH and GnRH are effective treatments for cystic ovarian conditions in cattle. A combined treatment of postpartum cattle having ovarian cysts with GnRH and $PGF_2\alpha$, usually results in ovulation within a 30-day period.

Faulty management practices that result in inseminations at improper times are still major factors in causing infertility in our animal populations. These practices include such things as re-breeding animals too soon following parturition, inadequate heat checking, poor record keeping, inadequate feeding during the postpartum period, and improper handling of either semen or embryos. Most of the managerial factors associated with maintaining optimal fertility are well understood and the problem now is one of educating cattlemen, inseminators, veterinarians and others associated with management practices, rather than a lack of fundamental research.

A number of reproductive diseases, including Vibriosis, Brucellosis, Leptospirosis, Trichomoniasis and mycotic infections contribute to infertility. These diseases are covered in other sections of this symposium and will not be discussed here.

2. Future Developments

Perhaps the major limitation to progress in reducing the high embryo mortality rates that occur in our farm animals is our lack of knowledge concerning the mechanism(s) by which the embryo signals its presence, causes maintenance of the maternal corpus luteum, and disarms the maternal immune mechanisms, which otherwise would recognize the embryo as a "foreign body" and destroy it. Several attempts are now being made to identify the embryonic luteotropin responsible for this signal to the maternal corpus luteum and it is expected that the compound(s) responsible will be isolated and characterized in the near future. Once this compound(s) is identified and becomes readily available it may be possible to use it to reduce early embryo mortality. Furthermore, isolation and identification of this "early pregnancy factor" may lead to development of an early pregnancy test for use in farm animals, especially cattle.

Recent findings show that a pulsatile pattern of LH secretion develops during the postpartum period and the development of this pattern is inhibited by lactation, suckling and possibly by stresses that cause the release of large amounts of endorphins and adrenocorticoids. The first postpartum ovulation often results in a corpus luteum that functions for only a few days and is thus incapable of supporting an embryo. Since pulsatile LH release occurs in response to pulsatile GnRH secretion from the hypothalamus, it would appear that administration of GnRH in a pulsatile manner may be effective in shortening the postpartum interval and hastening the time of first estrus and ovulation. Thus we may expect the development of methods for administration of GnRH and/or LH in a pulsatile fashion during the postpartum period, as well as the development of more potent, longer acting GnRH analogs. These developments, along with the previously discussed methods for inducing estrus in the postpartum period, should contribute to solutions of the problem of prolonged postpartum anestrus in cattle.

An important limitation to reproductive efficiency in sheep is the seasonal nature of the occurrence of estrus. This seasonality appears to be controlled primarily by photoperiod. The pineal gland and its major product, melatonin, have been implicated in several systems controlled by photoperiod. Thus, it may be possible to manipulate seasonal breeding by altering the output of melatonin by the pineal gland or influencing the effects of melatonin on the hypothalamic-pituitary-ovarian axis. Melatonin administration mimics the effects of exposing an animal to a dark period and could therefore replace light control as a means of controlling seasonality of reproduction in the ewe. Melatonin is highly active in ruminant animals and doses as low as 2 mg per day will maintain blood levels at the high levels characteristic of night-time for 7 or more hours. Clearly, intense work in these areas of infertility is fully justified and offers a good chance of improving reproductive efficiency in farm animals during the next ten years.

D. Genetic Engineering Techniques for Farm Animals

1. State of the Art

A remarkable series of recent developments suggests that a number of manipulations of the early embryo that may eventually have profound effects on the animal industries can now be carried out. Recombinant DNA technology has made it possible to prepare purified genes and inject them directly into recently fertilized eggs. These developments are, to a large degree, dependent upon the fortunate circumstance that, in mammalian embryos, the male and the female pronuclei do not fuse for 6-8 h after fertilization. It is possible to take advantage of this fact and inject recombinant DNA into one of the pronuclei, so that it is then incorporated into the embryo when the nuclei fuse. The most remarkable experiments in this area are those reported by Palmiter et al. (1983) in which both the rat and human growth hormone genes have been injected into fertilized mouse eggs, which were then transferred to recipient mice. These genes were expressed in a number of the resultant offspring, which grew significantly larger than control mice.

Previously, other workers introduced the genes for human β-globulin and insulin and rabbit β-globulin into mouse embryos, using the same techniques (See Hansel and Milvae, 1984, for review). These genes not only directed the synthesis of these compounds in the resultant mice, but were then transmitted through the germ line to a second generation of mice. Thus, it may be possible to disseminate new genetic material into cattle, sheep and swine populations by embryo transfer techniques. Indeed, attempts are now underway at a number of research laboratories to introduce these genes, and others into recently fertilized cow, sheep and pig ova.

A number of mammalian hormones, including insulin and bovine growth hormone, can now be produced cheaply and in large quantities in the laboratory by the application of recombinant DNA techniques. Administration of bovine growth hormone produced by recombinant DNA technology has been shown to increase milk production in dairy cattle by about 15%. Hormones controlling reproduction can also be produced in this way and administered directly to animals to improve reproductive rates and, conceivably to increase resistance to reproductive diseases. However, at present there are no really good slow release vehicles for long-term administration of exogenous peptide hormones to farm animals.

2. Future Developments

Despite the recent spectacular breakthroughs in introducing new genes into mouse and rabbit embryos, we expect developments in this area in farm animals to occur at a relatively slow rate. In a very real sense, these developments are dependent upon those occurring in the areas of estrous cycle regulation, superovulation and embryo transfer, since the latter techniques are necessary parts of most experiments in genetic engineering.

A great deal remains to be learned about the mechanisms involved in expression and transmission of introduced genes. The frequency with which transgenic animals are produced and the success rates in obtaining expression of the integrated genes have been extremely variable. In some cases expression has been demonstrated, but in inappropriate tissues and the unusual patterns of gene transcription have been shown to be heritable traits. The kinds of problems that must be solved are illustrated in the recent experiments of Palmiter, et al. (1983). These workers fused the promoter region of the mouse gene for metallothionein to the structural gene coding for human growth hormone. These fusion genes were then introduced into mice by microinjection into the male pronuclei of fertilized eggs. Seventy percent of the mice that incorporated the fusion genes had high concentrations of human growth hormone in their serum and grew significantly faster than control mice. Increased growth hormone secretion was induced by addition of zinc to the diet, a procedure that normally induces metallothionein gene expression. However, it was found that the human growth hormone was produced in the same tissues as the metallothionein (brain, liver and kidney), instead of the pituitary gland. Indeed, the pituitary gland cells (acidophils) that normally produce growth hormone were damaged by the high levels of circulating growth hormone. Thus long-term stimulation by hormones produced in this manner is likely to be associated with abnormalities in the production of other hormones and it becomes important to develop methods for turning the introduced gene on and off, so that its effects can be rigidly controlled and limited to critical stages in the animals' life.

Nevertheless, the outlook for introducing genes that control reproductive functions into farm animals by these methods is good. Research with the Booroola strain of the Merino sheep provides an excellent example of what could be achieved in this area. The Booroola is one of the most fecund sheep in the world; its fecundity is due to a single major gene, which decreases the production of inhibin by the ovaries and increases the level of circulating follicle stimulating hormone (FSH), thus causing an increase in the number of ova shed (Cummins et al., 1983). The gene also causes a deficiency of the lysosomal enzyme, neuraminidase in the liver, thus reducing the rate at which FSH is degraded. These three effects result in multiple ovulations in the Booroola, much as is caused by injections of exogenous FSH and LH. However, these animals do not become immune to their own hormone, as they tend to do in response to exogenous hormones, and there are no problems of developing a "delivery system". Indeed, the animal appears to have lost its ability to regulate its ovulation rate to a level that is compatable with its own uterine and maternal capacity.

Incorporation of this gene into the genomes of other breeds of sheep throughout the world seems a desirable goal. Therefore, attempts are being made to isolate the DNA for the Booroola gene and to produce transgenic sheep that have incorporated the gene into their chromosomes. Worldwide, a development of this sort could greatly increase the efficiency of production of high quality wool.

At present, relatively little is known about the chromosomal integration of foreign DNA, its retention throughout embryonic development until birth and, ultimately its expression without disruption of embryogenesis. Thus, it is important to foster research efforts on these fundamental processes.

III. SOME MAJOR BREAKTHROUGHS NEEDED

A. Slow Release Vehicles for Administering Peptide Hormones and Drugs

The lack of highly effective methods for administering certain hormones and drugs is a major deterrent to the widespread use of these compounds to promote growth and improve production and reproduction. For the most part, such compounds as GnRH, LH, FSH, and growth hormone are not orally effective, and are not efficiently released from silastic rubber and other polymer implants as is the case for steroid hormones. At present, these compounds must be injected daily, or more frequently, to be effective and this is usually impractical. Indeed, it now appears that some hormones (GnRH, LH) should be administered in a pulsatile manner to be most effective. New approaches to these problems, involving cooperative efforts of physical chemists, engineers and physiologists, are needed.

B. Improved Cell Culture, Separation and Transfer Techniques

It is becoming increasingly evident that most important physiological events depend on the combined effects of secretions of a number of different cells. Often, these secretions are locally acting substances that have short half-lives. These mechanisms are particularly evident in studies on the placenta and the corpus luteum. Thus, it is necessary to develop efficient methods for separation and culture of specific cell types and for their subsequent transfer to recipient animals. For example, it may be possible to separate and culture the growth hormone producing cells from pituitary tissue and, subsequently implant these cells in growth chambers into animals at critical stages of their growth period. The chambers containing the growth hormone producing cells could then be removed when additional growth hormone production was no longer desirable.

C. Development of Assay Techniques That Do Not Require The Use of Radioactive Substances

Radioimmunoassays are the cornerstone upon which our knowledge of hormone actions and interactions is built. Unfortunately, these assays require the use of small amounts of radioactive labels and the costs of disposing of the resultant radioactive waste have become prohibitive. Other methods, giving equal precision and repeatability and suitable for assaying large numbers of samples at low cost must be found. Enzyme-linked immunoabsorbent (ELISA) assays are a partial answer to this problem, but other, more precise methods are needed. For example, perfection of these techniques would foster the development of "cow-side" pregnancy tests, or tests to determine the optimum time for insemination.

D. New Sources of Funding for Basic Research

The traditional funding of animal science-related projects through small Hatch Act grants is clearly inadequate to support the kinds of research needed to accomplish the breakthroughs described. Indeed, a number of the significant findings described above on control of ovarian functions, embryo transfer and genetic engineering of embryos have resulted from projects funded mainly from non-agricultural sources. Additional funding for research on the biology of reproduction is clearly needed in order to capitalize on the recent breakthroughs and to make new ones. New methods of distributing these funds, so that they will be more effectively utilized, are also needed.

IV. INSTITUTIONAL ARRANGEMENTS

It seems clear that new institutional arrangements are also needed to accomplish the kinds of research outlined above. A degree of cooperation between state and federal research agencies and industrial laboratories that has not previously been achieved is required. The major drug companies and the new biotechnology industries must also play important roles in the developments that have been described. Furthermore, applications of the technologies being developed in the areas of estrous cycle regulation, superovulation and embryo transfer will need to be marketed through relatively large and efficient organizations, such as the major artificial breeding and embryo transfer companies. For example, marketing of estrous cycle regulation techniques through individual veterinarians is not likely to bring about effective large scale utilization of these techniques.

Centers, or institutes, probably located at major universities and funded jointly by industrial, state and federal efforts seem to offer the best arrangement for promoting research of the type needed. The Cornell Biotechnology Institute, funded jointly by a consortium of industries and New York State may represent the kind of research organization needed.

V. LIKELY TIMING OF COMMERCIAL INTRODUCTION

These research developments are capable of greatly increasing the rate of genetic progress in our domestic animals, but predictions of the time at which commercial applications of these developments will occur is difficult, since so many factors beyond our control are involved. Previous predictions have erred on the conservative side, and the increased economic pressures on the animal industries may operate to speed the application of some of the techniques described, since they will improve efficiency of production.

A. Applications Expected Within 5-Years

(1) Marketing of improved methods of estrous cycle regulation;

(2) Extension of improved embryo transfer techniques to a significant portion of the dairy and beef cattle and sheep and swine populations;

(3) Perfection of embryo freezing techniques;

(4) Perfection of in vitro fertilization techniques and;

(5) Development of a simple early pregnancy test for cattle.

(6) Perfection of a method for sexing embryos.

B. Applications Expected Within 10-Years

(1) Marketing of a complete animal breeding system based on improved superovulation, embryo storage, embryo sexing, embryo splitting and cycle regulation of the recipient animals;

(2) A marked increase in fertility of farm animals, due to application of results of studies on the early pregnancy recognition factor and on development of methods to shorten the postpartum anestrous period; and

(3) Separation of the X- and Y- bearing spermatozoa.

C. Applications Expected Within 15-Years

(1) Marketing of genetically engineered embryos, containing genes that will improve fertility and fecundity and result in improved rates of gain, carcass quality, and milk production in the resultant offspring.

VI. POTENTIAL PRIMARY IMPACTS

It is evident that if all of the technology now available were utilized, a new kind of animal breeding system could be put into operation within 10 years. If the estrous cycle regulation methodology now available were put into practice, artificial insemination could be extended to beef cattle, where it is not now widely used. If the combined advantages of the technology available for superovulating cows, collecting, processing and storing embryos were combined with the methods for estrous cycle regulation, artificial insemination in a significant number of our cattle, sheep and swine could be replaced by artificial inembryonation. Under such a system, embryos would be collected from superovulated animals, sexed, stored frozen and, later placed in the uteri of large groups of recipient animals whose estrous cycles would be coordinated with those of the donor animals.

The output of these new technologies will be in the form of new products and new processes, ranging from new and improved methods to store embryos in the laboratory to completely integrated animal breeding systems. Drug companies, genetic engineering companies, equipment manufacturers, veterinarians and extension workers will all be affected. A major effect of these developments will be to increase the efficiency of production of animal products. The efficiency of milk and meat production will probably be improved by at least 10%. Fewer animals will be used to produce the required amounts of animal products and less labor will be required.

REFERENCES

Cummins, L. J., O'Shea, T., Bindon, B. M., et. al., "Ovarian Inhibin Content and Sensitivity to Inhibin in Booroola and Control Strain Merino Ewes," J. Reprod. Fertil. 67:1-7, 1983.

Fitzhugh, H. A., Hodgson, H. I., Seaville, O. J., et. al., in The Role of Ruminants in Support of Man, Winrock International, April, 1978.

Hansel, W., "Future Agricultural Technology and Resource Conservation: Beef and Dairy Cattle, Sheep, Goats, Swine and Poultry," in RCA Symposium: Future Agricultural Technology and Resource Conservation, Center for Agricultural and Rural Development, B. C. English, J. A. Maetzold, B. R. Holding and E. O. Heady, eds. (Iowa State University, Ames Iowa, 1984).

Hansel, W., and Convey, E. M., "Physiology of the Estrous Cycle," J. Animal Sci. 57(Suppl.2):404-424, 1983.

Hansel, W., and Milvae, R. A., "Compounds for Regulating Reproduction in Domestic Animals," in Agricultural Chemicals of the Future, (BARC Symposium, Number 8), J. L. Hilton, ed. (Rowman and Allanheld, 1984).

Palmiter, R. D., Norstedt, G., Gelinas, R. E., et. al., "Metallothionein-Human GH Fusion Genes Stimulate Growth in Mice," Science 222:809-814, 1983.

Seidel, G. E., "Superovulation and Embryo Transfer in Cattle," Science 211:351-358, 1981.

Seidel, G. E., "Mammalian Oocytes and Preimplantation Embryos as Methodological Components," Biol. Reprod. 28:36-49, 1983.

Background Paper No. 3

Regulation of Growth and Development

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REGULATION OF LIVESTOCK GROWTH AND DEVELOPMENT

Summary

The rate and composition of growth is a critical factor in determining the cost of producing livestock products. While much is known about genetic and nutritional variables that influence animal growth, much less is known about the hormonal, cellular, and metabolic mechanisms that determine how and at what rate nutrients are partitioned into the growth of muscle, fat, and bone, the tissues of major concern. An understanding of these fundamental mechanisms is needed to provide the foundation for applying new technologies to the development of products to improve the rate, efficiency, and composition of animal growth.

The potential applications of genetic engineering, cloning, and immunology for the improvement of growth in food-producing animals are many. For example, recombinant DNA technology is responsible for providing sufficient quantities of bovine and porcine growth hormone so that scientists can now determine its role, mode of action, and potential use when administered to animals used for producing meat and milk. In the future, this kind of research may also lower the cost of beef production by permitting the production of large market cattle of desirable composition from small cows, which have lower maintenance costs than large cows. It also seems likely that biotechnology will give rise to new products that can alter the inherent mechanisms of muscle protein and adipose (fat) tissue accretion so that the efficiency of meat production is improved by the conversion of more nutrients into lean meat and less nutrients into fat. Such a development would be in keeping with consumer demand for lean, but highly palatable meat at a reasonable cost, and medical recommendations that the U.S. consumer reduce the intake of calories from dietary fat.

In addition to the application of biotechnology for the improvement of food-producing animals, there are also opportunities for advances that involve the physical sciences. These include the need for more rapid, accurate, and economical ways of maintaining the identity of animals through the time of slaughter, and for determining the composition of the living animal and its carcass. Improved methods of identifying mammalian meat animals would be a basis for a national record system. This system would benefit producers, packers, regulatory agencies, and consumers, since it could provide information concerned with marketing, carcass merit, disease, and residue monitoring programs.

A quick and accurate assessment of body composition would not only improve livestock production data and marketing procedures, but would be an example of new technology that could also be used to address human concerns about body weight and obesity. Current procedures used for determining body composition in livestock are too slow, inaccurate, or expensive for adoption by the industry. As a result, the real value differences between animals of low and high carcass merit, as affected by fat content, are normally not fully realized in the market when animals are sold live.

The implications of applying these kinds of technologies to improve the production efficiency, composition, and consumer cost of animal products are numerous. These considerations include the more efficient use of livestock feeds, possible changes in crop production priorities, improved composition of animal food products, improved production practices from more complete animal records, and implications related to human health. The application of these technologies will be dependent upon understanding the fundamental principles or mechanisms involved in each major research area.

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I. Introduction

The growth of animals and their developmental changes represent one of the most important events associated with the production of livestock and their products. The rate of growth and development is known to be influenced by genetic as well as environmental factors, such as nutrition, disease (clinical and subclinical) and temperature. However, much less is known about the cellular, hormonal and metabolic influences and interactions on the growth and development of bone, muscle and adipose tissue, which are the major tissues of concern to meat production (Trenkle and Marple, 1983; Mersman, 1982; Pond et al., 1980).

Although bone is not consumed, its growth determines the size of the animal, and either abnormal or less than optimum development can adversely influence production efficiency. For example, the ultimate mature skeletal frame size of animals is quite variable depending upon their genetic and environmental background, (Koch and Alego, 1983), and skeletal problems frequently decrease rate of gain or shorten the productive life of breeding animals (Fredeen and Harmon, 1983). Muscle is the primary goal of meat production but the quantity and efficiency of its growth is highly variable among animals of a given species. For high quality meat, some adipose tissue in the carcass is desirable. However, many animals partition excessive dietary energy into adipose tissue and produce carcasses that are excessively fat (Allen et al, 1976; Berg and Walters, 1983). Excess fat is not only unnecessary and inefficient to produce but makes a significant contribution to the high levels of fat consumed by the average American (Allen and Mackey, 1982).

II. Current State of Art

The general characteristics of animal growth and the production variables that influence growth and carcass composition of animals are quite well established. In addition, the ideal animal or carcass can be defined in relation to producer and consumer needs (Berg and Walters, 1983). However, the mechanisms which control growth and development at the tissue and cellular level is only beginning to be elucidated. The recent advances in gene splicing, recombinant DNA technology, immunology and other major biological advances not only provide the necessary tools to more fully understand "growth biology," but provide the potential for new products that would improve the efficiency of producing meat, milk and eggs (Anon. 1984). Prior to the advent of these new biological advances, a greater understanding of growth biology was primarily of academic interest. However, this situation has changed because there is potential for modifying and improving animal growth parameters through products and methods that were barely conceivable a decade ago. For example, the recent production of growth hormone by recombinant DNA technology now provides sufficient quantities of this hormone to study how it influences the growth and development of livestock species and the products from them (Morris, 1981) and allows consideration of its use in production. Prior to its production by recombinant DNA technology, growth hormone had to be isolated from pituitaries and its administration was primarily limited to studies in small animals such as rodents.

There is presently much academic and commercial interest in expanding the mechanistic understanding of growth biology. For example, a greater understanding of cellular proliferation and differentiation of bone, muscle and adipose tissue is necessary to enhance the chances of being able to manipulate the growth and development of these tissues with techniques common to the "new biology" (Allen, 1984). In another area, little is known about cell receptors in these tissues of livestock species. Until more is known, it will be difficult to maximize efforts for possible hormonal and immunological control of growth in food-producing animals.

III. Major Lines of Research

A. Muscle and Adipose Tissue Accretion

Protein is the major constituent of interest in muscle, and it would be desirable to have a maximum conversion of dietary protein and non-protein nitrogen into muscle or lean meat. In contrast, neutral lipids are the major constituent of adipose tissue. It would be desirable to minimize the conversion of dietary energy into carcass adipose tissue. The accretion rate of both muscle protein and neutral lipid is determined by the differences in the rates of synthesis and degradation of protein and neutral lipids. For example, it has been reported that about 75% of the daily muscle protein that is synthesized in the rapidly growing pig is necessary to replace the muscle protein that is degraded. From an energy point of view, this is very costly and is probably a major factor in contributing to the differences among animals in the efficiency of muscle growth. Some of the cellular and metabolic variables that contribute to the differences in the rates of synthesis and degradation of muscle protein and adipose tissue lipids are known. However, much less is known about the hormones, serum factors and cell receptors that control the rates of synthesis and degradation in the living animal. The large variation in quantities of muscle protein and adipose tissue lipid among animals of different genetic background suggest that there are major differences in factors controlling the partitioning of dietary protein and energy into muscle and adipose tissue. When these factors are identified or mechanisms are more fully elucidated, it seems likely that they will be subject to manipulation by new products or alteration by techniques common to the "new biology."

B. Hormones, Serum and Tissue Factors Important to Growth

We have a very limited understanding of the hormones and serum or tissue factors that control cellular proliferation, differentiation and growth of bone, muscle and adipose tissues. For example, until rather recently growth hormone was believed to act directly on muscle growth. It is now rather well established that growth hormone has an indirect effect on muscle through somatomedins or other factors produced in the liver and possibly other tissues or cells. For example, Urist et al., 1983 reported that bone produces a factor which is involved in regulating the proliferation of cells important to bone growth. Other investigations are underway with tissue culture systems to determine if there are serum factors which enhance or inhibit the growth and/or differentiation of muscle and adipose cells. Studies such as these will enhance our understanding of the control of tissue growth and provide new possibilities for products that could be utilized to improve animal growth and carcass composition. It is well known that animals of a given livestock species vary widely in their mature body or skeletal frame size. For numerous reasons, including growth rate and carcass composition, it is currently desirable to have market cattle of quite large frame sizes. At the present time such cattle come from parents of similar skeletal size. However, a major cost of beef production is the maintenance cost of the cow, which is higher for large cows. Conceivably in the future, it may be possible to utilize small cows to produce offspring and then administer products which would postnatally increase their skeletal frame size and achieve a growth rate well beyond the genetic potential of the parents.

Using gene-splicing techniques a larger mouse has been genetically engineered (Palmiter et al., 1983).

C. Immunological Alteration of Animals

As more information on the mechanisms important to growth become known, it is very likely that some of these can be altered by immunological approaches. This is not only evident for some animal diseases but also other areas. For example, the production systems in the U.S. for market cattle, swine and sheep do not utilize the more rapid gains and increased leaness common to intact males compared to castrated animals (Seideman et al., 1982). The reasons for this are related to more difficulty in managing mature males as well as concerns related to meat quality. There is work currently underway to develop satisfactory techniques for immunological castration (Schanbacher, 1982). When perfected, such a technique would eliminate the need for physical castration at a young age and allow the animal to receive the additional growth stimulation from its own testosterone. At a time later in the life of the animal, immunological castration could be performed and may improve management of the animal and help assure that the tenderness of the meat is maintained, since this can become a problem in intact males. Other examples related to cell receptors or other hormones important to animal growth will most likely become evident as new information becomes available. Some possibilities include immunoneutralization of somatostatin, a growth hormone release inhibiting factor (Spencer, 1983), and decreased muscle protein degradation.

D. Measuring Body Composition and Animal Identification

Research on animal growth and the marketing of livestock in the U.S. suffers from a lack of precision in methods used to determine the body composition of living animals in a quick and economical way. As a result, many livestock are still purchased on the basis of averages for animals of a given type. Consequently, the producer of the most desirable animals is generally not paid enough, whereas the producer of fatter animals is paid too much (Parker and Pope, 1983). In animal growth research, there is frequently a need to follow the changes in composition of the living animal during a trial. With new engineering advances, such as computerized axial tomography (CAT) and nuclear magnetic resonance (NMR) scanners, it would seem that a more satisfactory way could be developed to determine the composition of living animals. Such techniques would also be helpful to humans interested in weight control and body composition. For livestock species, this developmental effort would need to be interdisciplinary and involve the expertise of both engineers and animal scientists.

In addition to the research and marketing needs for more accurate assessment of body composition in animals, there are multiple reasons for a system in which animal identity can be maintained from production through marketing. These include the tracking of animals for production, marketing and disease records, and the monitoring that is necessary at the time of slaughter for tissue residues and product safety. An electronic monitoring system would offer the advantages of speed, accuracy and direct linkage to a computerized national system.

IV. Major Breakthroughs Needed

The major obstacle confronting all four lines of research that have been enumerated is the lack of necessary funding to accelerate the research that is needed. This has been especially critical for studies on livestock species where the cost of feeding and maintaining the animals for longer times is greater than for rodents. In many of the studies with livestock species, an expensive carcass is lost because the drugs, hormones or isotopes that are administered during the course of the experiments are either not approved for use in food-producing animals or render the meat unwholesome for human consumption. As a result, granting agencies have tended to fund research projects with laboratory animals or which address human problems where livestock species might be the more appropriate models. With the advent of recombinant DNA technology and the possibility of new products, some industrial grants have recently been given to investigators interested in growth biology who are working with livestock species.

The lack of government funding has also created a shortage of people qualified to conduct research in the area of growth biology and who are capable of doing fundamental studies with livestock species. Currently the demand is much greater than the supply for such individuals in industry, university and federal research groups or departments. As a result, many jobs have either not been filled or have been filled by people with less than ideal training to do the necessary research. This situation would be improved by additional research funds and by money to support graduate and postdoctoral fellowships. A number of Animal Science Departments have recently initiated or expanded specific undergraduate or graduate courses in the growth biology area. In addition, the first interdisciplinary international symposium in this area was held in 1982 at the University of Guelph, and the second one will be held this summer at the University of Missouri. These are additional indications of keen interest in the area.

In addition to funding problems and an insufficient number of appropriately trained scientists, there are other obstacles that need to be addressed. One of the problems in studying animal growth is that it is a slow, continuous process with no notable outstanding or cyclic events such as those associated with the process of reproduction. Unlike humans, the pubertal growth spurt does not occur in livestock species. Thus, it is difficult to study bone, muscle or adipose tissue growth in short-term experiments, especially when the methods for accurately determining body composition are lacking. As an alternative, in vitro techniques, such as tissue culture, are being developed and utilized for fundamental studies where the emphasis is upon mechanisms. These kinds of studies are underway in some laboratories that are concerned with growth biology in livestock species. These in vitro techniques may also become useful in the future for identifying the growth potential of animals with superior genetic makeup. For example, blood serum tested in tissue culture may provide an early and improved method for assessing an animal's potential to produce lean meat. Such a laboratory test on a young animal would offer many advantages compared to the slow and costly methods currently used in assessing the merit of an animal for growth and body composition. Laboratory techniques of this kind would also be of benefit in providing more rapid and less costly methods for industrial screening of potential compounds for altering the rate and/or efficiency of animal growth.

During the last decade, many advances have been made in laboratory instrumentation and this does not appear to be a serious limitation, other than the cost of the equipment and expendable supplies such as isotopes, tissue culture supplies and disposable labware. However, as previously indicated, an improved method for determining body composition would be a major contribution to progress in research on growth biology, determining the actual market value of livestock, and addressing human concerns about body composition and obesity. Present technology that utilizes ultrasonics, specific gravity, electrical conductance, potassium^{40} (naturally occurring stable isotope used in measuring lean tissue), deuterium oxide, nuclear magnetic resonance and CAT scanners to estimate body composition are unsatisfactory for widespread use because of either insufficient accuracy, cost of equipment or the time required to make the measurement. Interdisciplinary teams need to be funded and challenged with finding a cheaper and more satisfactory method so that body composition can be quickly and accurately determined.

Additional funding for fundamental studies in the four priority areas identified is necessary if reasonable progress is to be realized. The current resources available for work in growth biology of livestock species are simply inadequate. New funding in the range of six to seven million dollars annually would have a significant impact in developing the necessary growth biology research centers. The grants should be sizeable, awarded on a competitive basis and many should be given to interdisciplinary teams capable of working directly with livestock species.

V. Institutional Arrangements

Since a primary need is for fundamentally trained people with an understanding of research needed to alter or enhance the growth of livestock species, the role of Departments of Animal Science in this effort is critical. Some of these departments are best positioned to fulfill the need for such human resources because they are involved with both fundamental and applied research in this area of animal agriculture.

As a result, they have the animal resources, teaching programs, familiarity with the needs and some of the faculty and physical facilities to do such work. Many of these departments are capable of assembling interdisciplinary teams of scientists that would involve expertise from other departments in land grant universities.

It is expected that other university groups will continue to make needed fundamental contributions to the understanding of growth as it relates to tissue metabolism, receptors and hormonal control. However, these groups are generally unfamiliar with the needs and real implications of such work to improve meat production in livestock species. It is also unusual when these groups do research with livestock species with a primary objective of improving these species for food production. This is not unexpectd since this is a primary responsibility of land grant university departments and there has never been any significant federal or industrial grants to encourage their interest in such an objective (Anon., 1982). With appropriate grant incentives and some increased awareness of fundamental work that is needed in livestock species, some groups in private universities could make greater contributions to advancing the understanding of growth biology in these species.

Among federal government laboratories, certain USDA-ARS laboratories should continue to be involved in this area of research. However, efforts in fundamental growth biology research to date are somewhat fragmented among different locations with a few scientists at each of three laboratories. In general, these laboratories are relatively well equipped and the small size of the groups would be greatly enhanced by more postdoctoral fellows. This problem has been perceived by others and is apparently not unique to the area of growth biology (Anon., 1982).

Some group consolidation and priority of importance to animal growth biology in the USDA-ARS system would be desirable if the expected contributions from these laboratories are to be achieved. It would be unfortunate if this did not occur because the potential for significant contributions from this component is great.

As indicated previously, the advent of new biotechnologies has provided a significant new tool and reason for private industry to become interested in animal growth biology and the development of new products. The recent increase in industrial interest has contributed in a major way to the need for additional information and scientists in this area. Industry has responded by establishing new research groups that are conducting a cross-section of fundamental, developmental and applied studies. In addition, some industrial grants or contracts have been awarded to university departments for more fundamental studies. A recent paper discusses the need for greater interaction among federal, university and industrial laboratories in conducting research (Keyworth, 1983).

VI. Likely Timing of Commercial Introduction

There are a variety of considerations which will influence the commercialization of new products or technologies from the major lines of research that have been enumerated here in relation to animal growth and composition. The following are some examples of such considerations.

Base of Fundamental Knowledge

Commercial product development in this area is quite dependent upon an understanding of the biological mechanisms important to animal growth and body composition. Without sufficient information of this kind, the probability of successful products is lower and commercial development ventures are less likely to occur. Thus, the recent development of new biotechnologies, such as gene splicing and recombinant DNA techniques, provides a driving force for expanding the fundamental knowledge base so that this kind of technology can be more rapidly applied (Gomory, 1983).

Market Potential for a New Product

This consideration is complex. It involves projections into the future that may be altered by new competing products, government policies and regulations, development cost and time to gain government approval, degree of acceptance or penetration into the market place, and the potential for domestic and international markets. For example, a unique product that reduces the cost of producing broiler chickens will generally have a more rapid penetration in its market than a product for feeder lambs that gives a similar reduction in production costs. In part this example is related to the major differences in the way these two industries are structurally organized relative to unit size and degree of integration.

Patent, Regulatory and Other Government Policies

The ability to patent and obtain regulatory approval for use in one or more species is a critical aspect to encouraging commercial investment and development of products related to growth biology. If this is not a likely potential, it does not generally make good business sense for a company to take the risks in fully developing products that cannot be protected for a reasonable period of time from similar products in the market place. This is one of the critical issues of concern as universities consider how they can cooperate with commercial companies and yet maintain the freedom to pursue new knowledge and publish scientific information (Anon., 1984). In another area, government policies should assure that new products can be safely used in producing livestock products for human use, but yet not be so excessively restrictive as to discourage development. Regulatory policies concerned with using growth-promoting products that have been approved singly, but not in combination with other products need to be carefully evaluated in terms of their justification. It is highly probable that products which influence different biological mechanisms concerned with animal growth can be used safely in combination and produce additive benefits.

With the above considerations in mind, the estimates for the time for commercialization of one or more kinds of products in each of the lines of research are given in Table 1.

In addition to the previous general considerations that have been discussed with regard to the kinds of technologies enumerated in Table 1, there are some more specific examples that would have major effects in encouraging the development of these technologies. Some examples are as follows.

Table 1. Estimated Time For Commercial Introduction of Products or Technologies Related to Growth and Composition of Livestock

Research Lines	Primary Species	Estimated Timing of New Product Introduction
*Increased Muscle Protein and Decreased Fat Accretion in Carcasses	Cattle Sheep Swine Poultry	1986-1992
*Manipulation of Skeletal Size	Cattle Sheep	1995-2000
*Immunological Alteration of Natural Hormone Production	Cattle Sheep Swine Poultry	1988-1995
*Improved Efficiency Through Decreased Muscle Protein Degradation	Poultry	1995-2000
*Reproduction of Genetically Engineered Animal with Improved Growth Traits	Poultry	1995-2000
*New Technology for Animal Identification	Cattle Sheep	1988-1995
*New Technology for More Accurately Determining Body Composition	Swine	1995-2000

- During the last decade or so, the red meat industry has had some major changes in terms of plant location, adoption of new technical and marketing technology, and more innovative approaches to this industry (Koch and Alego, 1983; Breidenstein and Carpenter, 1983). During this time there have been changes in the USDA beef and lamb grading systems and discussions about a more meaningful USDA pork grading system. All of these changes or discussions have been in part related to decreasing the emphasis or credit that is given to carcasses with more fat. It is anticiapted that this trend will continue because of the increasing health-related concerns about animal fat in the human diet. Such a trend will increase the need for meat animals to partition more nutrients into lean meat and less into fat, while maintaining eating quality. This will also place greater emphasis on identifying variation in carcass composition and achieving appropriate price differentials. A major de-emphasis of marbling (intramuscular fat) in the USDA beef quality grades is an example of a change that would accelerate the need for some of the technologies in Table 1. In recent years, there has also been increasing concern about the quantity of fat present in the carcasses of broiler chickens (Allen and Mackey, 1982).
- Consumers are increasingly concerned about the safety of their food products. This trend will continue and will require that the industry and government regulatory agencies work together in monitoring meat for chemical residues and prosecuting producers that are in violation. Such a program would be greatly assisted by a standardized system of animal identification.

- The adoption of the technologies in Table 1 that involve the use of peptide hormones such as growth hormone, would be assisted by more information on hormone implant technology concerned with the stability and release of peptide hormones when implanted in animals. For example, research and development is needed to provide growth hormone implants which ensure that the biological activity of the hormone is maintained over a reasonable period of time, and that the proper level of the hormone is released. Studies on continuous compared with pulse release of peptide hormones are needed to determine the most appropriate release pattern to elicit animal response. For example a pulse release may be necessary to prevent "down regulation" of growth hormone production by the animal's pituitary gland. Further advances are needed on this kind of implant technology.

VII. Potential Primary Impacts

The research lines enumerated in Table 1 and other related possibilities will come about through the development of new products that either improve animal growth, determine body composition or improve upon the present methods for maintaining the identity of animals. Some of the potential impacts for each of these are as follows.

Animal Growth Improvers - As indicated in Table 1, there are many different methods or products that could theoretically improve animal growth. For these products to be accepted by producers, they must lower production costs by increasing rates of gain, decreasing feed to gain ratios, improving carcass composition or, in the case of breeding females, reducing maintenance costs. The overall impact of such changes would be a reduction in the amount of feed necessary to produce a given quantity of animal products such as meat, milk, eggs, wool and by-products. Thus, animal feed resources would be used more efficiently in producing animal products. The ultimate benefit would be to the consumer because the cost of production would be reduced and producers who do not adapt the technology would be at a disadvantage relative to cost of production. The feed savings that are realized could be used for additional animal production, if demanded by the market, made available for export or some of the land presently used for producing animal feedstuffs could be used for other crops. In some cases, these new products may encourage the use of more land for grazing cattle or sheep by making the returns per acre more competitive with crop production. Thus, some land that is now marginal for crop production could be returned to grasslands and help alleviate some concerns about pollution of streams and loss of productivity resulting from soil erosion.

Body Composition Determination - This area is the one of great need for both the livestock industry and human medicine, but may be the most difficult to resolve in terms of relative accuracy at a reasonable cost for equipment of the technologies discussed in this paper. However, if such a technology were developed and adopted it would remove much of the guesswork that is involved in quickly determining the real value of market livestock. Such a technology could improve the pricing efficiency at the market and provide producers with greater incentive for producing animals of the correct composition relative to demand. In addition to the use of such technology for market livestock, it would also be a valuable tool for improving the selection of breeding animals and for following the composition of animals during growth research studies. The feed used for the production of excessive beef fat in the U.S. has been estimated (Allen et al., 1976) to approximate the entire corn crop of Iowa. Many cattle that are fed to USDA Prime or Choice quality grades have excessive deposits of subcutaneous and internal body fat. In contrast, there are cattle which make these quality grades and contain very little excess fat in these depots. Thus, technology which assists in more accurately identifying the more desirable breeding or market animals relative to body composition would help decrease the production of animal fat and assist in reducing the dietary calories in the U.S. human diet that are derived from fat.

Animal Identification - The technology that is envisioned for this topic would ideally maintain the identity of the animal or its carcass from the time of birth until the carcass is inspected and graded. Such a technology should be accurate, inexpensive and lend itself to automation. Adoption of such a system would provide the basis for a national record system that would be useful for producers, meat packers and regulatory agencies. This system would be useful in programs concerned with evaluating sires, monitoring diseases and residues, relaying carcass information to livestock breeders and feeders, and in assessing the true value of livestock at the market. Unlike the dairy farmer who uses the Dairy Herd Improvement (DHI) records as a management tool in deciding which bulls to use and which cows to cull to improve milk production, the producer of meat animals frequently knows very little about the real carcass merit or value of the animals produced. This is because it is presently very difficult to relay information back to the people who would be most interested in it because the identity of the animals is not readily maintained. Similarly, when violative chemical residues are found in animal tissues at the time of slaughter, it is frequently difficult to readily identify the production origin of the animals. This situation weakens the role of the regulatory agency by increasing the cost of the monitoring program and delaying the correction of problems when they do occur. Thus, there are multiple needs and reasons to justify a technology that improves upon the present system of tracking the origin and identity of meat animals.

VIII. References

Allen, C. E. and M. M. Mackey. 1982. Compositional Characteristics and the Potential for Change in Foods of Animal Origin. In: Animal Products in Human Nutrition, D. C. Beitz, Ed., Academic Press. p. 199-224.

Allen, C. E., D. C. Beitz, D. A. Cramer and R. G. Kauffman. 1976. Biology of Fat in Meat Animals. North Central Regional Res. Pub. No. 234.

Allen, C. E. 1984. Perspectives on Future Directions for Animal Growth Research and Lean Meat Production. Reciprocal Meat Conference Proceedings (In Press).

Anon. 1982. Science for Agriculture. Report of a Workshop on Critical Issues in American Agricultural Research, Winrock International Conference Center. Publ. by Rockfeller Foundation, N. Y.

Anon. 1984. Commercial Biotechnology: An International Analysis (Washington, D.C.:U.S. Congress, Office of Technology Assessment, OTA-BA-218, January).

Berg, R. T. and L. E. Walters. 1983. The Meat Animal: Changes and Challenges. J. Animal Sci. 57:(Suppl.2)133.

Breidenstein, B. C. and Z. L. Carpenter. 1983. The Red Meat Industry: Product and Consumerism. J. Animal Sci. 57:(Suppl.2)119.

Fredeen, H. T. and B. G. Harmon. 1983. The Swine Industry: Changes and Challenges. J. Animal Sci. 57:(Suppl.2)100.

Gomory, R. E. 1983. Technology Development. Science 220:576.

Keyworth, G. A. 1983. Federal R & D and Industrial Policy. Science 220:1122.

Koch, R. M. and J. W. Alego. 1983. The Beef Cattle Industry: Changes and Challenges. J. Animal Sci. 57:(Suppl.2)28.

Mersman, H. J. 1982. Direction and Utility of Growth Biology Research. Reciprocal Meat Conference Proceedings. 35:13.

Morris, C. E. 1981. Genetic Engineering: Its Impact on the Food Industry. Food Engineering, May, p. 57-69.

Palmiter, R. D., G. Norstedt, R. E. Gelinas, R. E. Hammer and R. L. Brinster. 1983. Metallothionein-Human GH Fusion Genes Stimulate Growth of Mice. Science 222:809.

Parker, C. F. and A. L. Pope. 1983. The U.S. Sheep Industry: Changes and Challenges. J. Animal Sci. 57:(Suppl.2)75.

Pond, W. G., R. G. Merkel, L. D. McGilliard and V. J. Rhodes (Ed.). 1980. Animal Agriculture-Research to Meet Human Needs in the 21st Century. Westview Press, Boulder, Colo.

Schanbacher, B. D. 1982. Responses of Lambs to Active Immunization Against Testosterone and Luteinizing Hormone-Releasing Hormone. Am. J. Physio. 242:E201.

Seideman, S. C., H. R. Cross, R. R. Oltjen and B. D. Schanbacher. 1982. Utilization of the Intact Male for Red Meat Production. A Review. J. Animal Sci. 55:826.

Spencer, G. S. G., G. J. Garssen and I. C. Hart. 1983. A Novel Approach to Growth Promotion Using Auto-Immunization Against Somatostatin. I. Effects on Growth and Hormone Levels in Lambs. Livestock Prod. Sci. 10:25.

Trenkle, A. and D. N. Marple. 1983. Growth and Development of Meat Animals. J. Animal Sci. 57:(Suppl.2)273.

Urist, M. R., R. J. DeLange and G. A. M. Finerman. 1983. Bone Cell Differentiation and Growth Factors. Science 220:680.

Background Paper No. 4

Animal Disease Control

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Summary: Disease Control

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Diseases of livestock are the greatest single deterrent to production efficiency. Animal health related problems and reproductive inefficiency limits the productive capacity of livestock enterprises to 65 to 70% of their potential. Estimates of the magnitude of these losses is in excess of $17 billion annually. These losses could be much greater if the major epidemic diseases such as foot and mouth disease, hog cholera, tuberculosis among others had not been controlled or eliminated. Research and implementation of research knowledge has led to holding losses at the level that faces the livestock industry today.

The methods used for control of disease and management of animal health changes as the structure of the livestock enterprise changes. Today large, labor efficient livestock enterprises such as confined swine raising operations, 500 to 5,000 milking dairy cows, feed lots containing 50,000 plus cattle and sheep, and poultry operations with 100,000 or more birds are relatively common. Introduction of a single infectious disease can have devastating effects. On the other hand, insidious losses in productivity occur because of poorly defined animal health problems such as reproductive inefficiency, neonatal death losses, mastitis, among other diseases. Reduction of animal health related production losses can be brought about by improved management schemes and application of biotechnology leading to more effective disease control.

The technologies which show the greatest promise for increasing production efficiency through a reduction in animal health losses are 1) data management and systems analysis, 2) application of rapid diagnostic tests, 3) selection for disease resistant strains of livestock, 4) genetic engineering of micro-organisms and embryos and immunobiology. The application of computer technology to livestock units with large numbers of animals makes it possible to efficiently keep animal health and production records.

Retrieval of data by veterinarians and farm managers will quickly identify animals that need vaccinating, checked for pregnancy or those that need to be bred. The data can also be used to identify those of low production efficiency because of disease and these animals can be eliminated from the herd/flock. Analysis of data may eventually be used to assist in making management decisions.

The recent advances that have been made in diagnostic methodology are providing for simple, rapid and accurate tests such as the enzyme linked immunoassay (ELISA). These tests can be either qualitative or quantitative and in many instances automated readers interfacing with computers provide highly reliable results. In some cases cow side tests are being developed. These tests are economical and they provide the veterinarian with immediate results upon which important management and disease prevention decisions can be made. These tests will improve the effectiveness of veterinary intervention.

Selection of animals or poultry for disease resistance is being undertaken. The recent advances in embryo transfer technology will facilitate the rapidity with which disease resistant strains of animals can be propagated. Examples of diseases with which disease resistance has been demonstrated include Marek's disease and leukosis in poultry, trypanosomiasis and mastitis in cattle, and a form of coliform diarrhea in pigs.

The major constraints which may delay the implementation of these technologies in the livestock sector include 1) funding for field studies, 2) commercialization of products by the biological and pharmaceutical industries and 3) cumbersome quality assurance requirements.

I. Introduction

Animal agriculture is the most important aspect of agriculture transferring the sun's energy to the energy needed for human sustenance. Most plant products must pass through animals before humans utilize the sun's energy and earth's minerals. Animal products in the American diet provide two-thirds of the protein, phosphorus, and substantial amounts of essential vitamins and other minerals. Productivity of animals has increased dramatically with abundant natural resources and low-cost energy. Much of this success is the result of applying technology developed through research. Improved animal health has contributed significantly to increased productivity, through the control or elimination of many devastating diseases from our livestock populations. However, disease continues to be the single greatest deterent to maximizing livestock productivity. Only healthy animals and poultry can fulfill their genetic potential in the utilization of feed for efficient growth, reproduction and performance.

Disease limits production efficiency to 80% of the potential. These losses total $17.5 billion per year (Osburn, 1984). If the reduced reproductive efficiency are added to these totals, our food animal industries operate at about 65% or less of their potential production efficiency.

Although many serious major diseases have been eradicated or controlled, there are a number of subclinical diseases which lead to insidious losses. These diseases may cause decreased productivity, be debilitating, or predispose to other diseases. These diseases include infectious diseases such as leukosis and bluetongue; toxicoses such as mycotoxicosis and plant poisonings; parasitism; and metabolic diseases like ketosis. Certain important disease complexes, such as respiratory and enteric diseases and mastitis, continue to result in significant losses (King, 1981). These disease complexes remain important because of changing animal agricultural practices. The number of farms is greatly reduced and the number of animals and poultry on each farm is greatly increased. For instance, the number of hog producers has decreased from over two million in 1950 to approximately 400,000 today. With the larger production units there is a concentration of animals with fewer personnel to observe the animals. These practices increase the opportunities for spread of diseases and an increased loss in production efficiency. In order to provide the new strategies needed to address subclinical diseases and changing animal agricultural practices, veterinary medicine, the medical arm of agriculture, needs to develop rapid, accurate diagnostic systems, improve vaccines and management and develop disease resistant strains of animals and poultry.

A. Definition and Scope. Recent advances in data management and biotechnology provide the technical background to make significant breakthroughs in animal and poultry health and disease control. Animal biotechnology including genetic engineering for control of diseases and immunobiology offer the basis for improved diagnostic procedures and new improved disease control measures for health management systems.

B. Major Lines of Research. The principal lines of research addressing animal biotechnology and health management systems encompass the areas of 1) data management and systems analysis, 2) diagnostic methodologies, 3) selection for disease resistance, 4) genetic engineering of microorganisms and embryos, and 5) immunobiology.

II. Current State of the Art

The application of computer technology and data management is of particular importance for increasing production efficiency for the moderate and large sized livestock operations. The concentration of dairy cattle into relatively large milking herds (100-5,000 cows), confinement swine rearing operations and the poultry industry all have relatively large concentrations of animals managed by one individual and assisting personnel. Often the size of the operations are such that each individual is responsible for specified duties and no one individual handles all aspects of animal husbandry on a day-to-day basis. It is important that systems be developed that will allow the manager an effective and efficient means of assessing the well being of each animal. Computers and computer programs offer the livestock manager/owner this capability.

Computers and computer programs are now being used on many dairies, swine production units and in the poultry industry. Herd record keeping systems for animal health are being developed and refined for various production units. Examples of these programs now in operation include FARMHX in Michigan, and similar systems in New York and California (Mather, 1983). These record keeping systems may be linked with animal identification systems including radiotransmitters. Examples of the types of information that can be recorded for each animal include production record, feed consumption, vaccination profiles, breeding records, conception dates, numbers of offsprings, listing and dates of disease(s) and costs of medicants for treatment or prevention of disease. A review of printouts will allow the manager or veterinarian to quickly analyze a health profile for each animal. Bringing all of this information together will allow the veterinarian and manager of the livestock enterprise to plan for more cost effective disease control programs and to designate the duties such as vaccinations, pregnancy examinations, etc., which are to be carried out. These programs are now being applied and refined on a few farms. By 1990 many of the more progressive livestock producers will be using these systems and by 2000 these systems will be widely applied to nearly all of the cost efficent livestock production units. This concept in herd health management will change the manner in which veterinary medicine will be practiced.

Veterinarians will play a greater role in planning and implementing preventive herd health programs along with the livestock manager.

Recent advances in biotechnology include the use of rapid, accurate diagnostic systems. Already there are efforts, and in some instances animal-side test kits, for diagnosing different diseases. The use of enzyme-linked immunosorbent assays (ELISA) and the biotin-avidin systems allows for the tagging of antibody molecules which when reacted with antigen and another set of reagents causes a colorimetric change indicative of a specific reaction. These tests can and are replacing the highly sensitive radioimmunoassays which have been used to measure picogram amounts of antigens. The absorption of antigen onto paper (immunoblotting) followed by incubating immune serum tagged with biotin-avidin and enzyme-substrate causes a color change which provides for a highly specific test for diagnosing diseases. Similar tests can also be included in small kits to which serum can be readily applied and the test quantitatively measured in spectrophotometric readers. If desirable, the spectrophotometric readers can be interfaced with a computer where data is recorded and quantitatively analyzed.

Examples of ELISA systems for diagnostic tests include assays for hormones, detection of drug residues in milk or feeds, antibody detection for brucellosis, infectious bronchitis, infectious bovine rhinotracheitis, and parasites (Bolton, 1981; Farrell, 1981). The use of the ELISA test for the hormone progesterone may be used as a means of determining if a cow is pregnant (Stabenfeldt, 1984). Milk or serum samples collected 21 days following breeding of the cow would indicate that the cow is pregnant if sufficient measurable progesterone can be detected in the samples. Since this hormone is associated with maintenance of pregnancy at 21 days following breeding it can be used to make this diagnosis much sooner than can be done by manual palpation at 30 to 40 days following breeding as currently practiced. These test systems are being adapted to test kits that can be used at the side of the animal on milk samples. By reducing the interval that cows are not pregnant, it increases the production efficiency of the system.

Application of this same technology to a number of infectious diseases of livestock and poultry makes it possible to very rapidly and accurately diagnose the cause of the illness. Diarrheas caused by rotaviruses can be rapidly diagnosed by collecting and analyzing fecal samples (McGuire, 1982). As this technology continues to be adapted for various infectious diseases, the veterinarian will be in a position to more accurately and rapidly diagnose the cause of the disease problems. Currently, collection of samples and submission to diagnostic laboratories may require at least 24 hours to seven days or longer. The use of these new tests to expedite the diagnosis will assist the veterinarian by providing information on the cause of the disease and allow him/her to make rapid, sound decisions for treatment and for reducing the spread of the disease throughout the flock or herd.

Similar test systems are being commercially prepared for detecting antibiotic residues in milk. This is important for the dairyman to know so that he/she can get the animal back into the production line following treatment for mastitis. Contamination of bulk milk tanks with milk from one cow treated with antibiotics may result in a loss of thousands of dollars as all of the milk must be discarded.

The coordination of these test systems with the computerized herd health management program can be orchestrated to assist in management. For instance, use of hormone assays to diagnose pregnancy can be programmed so that on any given day all animals that are to have progesterone assays performed on milk samples are identified. The samples can then be obtained and the results read to determine the reproductive status of the cow. If the cow is not pregnant she could be listed as one to be bred. The computer and diagnostic technics are management tools aimed at mimimizing the losses that result from the more complicated livestock production units.

The fact that viral and microbial gene segments can be isolated, inserted into plasmids and cloned in other bacteria or fungi provides a means of preparing unique diagnostic reagents. Since bacteria and fungi multiply rapidly, the number of cloned foreign gene segments can be produced in large quantities in a short period. These genes when denatured seek complimentary structures and reanneal. This phenomena is the basis for identifying foreign microbial genes in cells and tissues. These highly specific genetic reagents can be used for sensitive and specific diagnostic tests. These tests have been used for identifying and diagnosing infectious bovine rhinotracheitis virus, bluetongue virus and pseudorabies virus (Ackermann, 1982; Roy, 1985; Squire 1985; Stott 1984).

Over the years, there have been a number of diseases for which no suitable solutions have resulted from research efforts. Examples of some of these diseases are mastitis, leukosis in chickens and cattle, external parasites in cattle, and coliform enteritis in swine. On the other hand, there are strains and/or breeds of animals that are resistant to these different diseases. The ability to identify gene markers and to selectively breed for the desirable characteristics will eventually have a major impact on disease control.

The recent advances in embryo transfer technology, gene insertion into embryos and amplification of the gene products are now coming into being. With this technology at hand, it will be possible to rapidly expand the number of desirable offsprings and thereby bring about major reductions in disease.

The new biotechnology has definite application to disease control. Generally, the most effective viral vaccines have been modified live virus or attenuated live virus vaccines. These vaccines can replicate in the host following vaccination and in most instances cause a very mild form of infection. The major concerns with these vaccines are 1) they can be a source of infection which can spread to other susceptible animals, 2) reversion to virulence may result in clinical disease, and 3) that potential foreign animal disease vaccines such as foot and mouth disesase virus vaccine cannot be safely administered to animals in countries where the disease does not normally occur. The use of recombinant DNA technology to make effective vaccines which are incapable of replicating in the host is a very desirable feature of these products. A vaccine for foot and mouth disease virus utilizing recombinant DNA technology has been accomplished. It should be possible to develop similar vaccines for other viruses such as bluetongue, pseudorabies, papilloma (warts), rotavirus and others which would have important applicability for disease control in the next 15 years (Pilacinski, 1984; Robbins, 1984). Recently, a gene coding for the envelope glycoprotein of vesicular stomatitis virus has been incorporated into vaccina (smallpox) virus and used to vaccinate cattle for vesicular stomatitis virus. Cattle vaccinated with this product were protected from challenge infections (Mackett, 1985).

Similar innovative vaccines for important bacterial diseases affecting animals are being tested at this time. Vaccines for different strains of salmonella have proved to be effective. These new vaccines contain live salmonella; however, they are genetically altered so that they cannot reproduce (Smith, 1984). Such innovative vaccines hold promise for control of important bacterial diseases.

Knowledge about the basic function of the immune system has been acquired over the last two decades. Application of basic principles learned during this time provides the basis for improved efficiency and effectiveness of the immune system. A few examples representative of ways in which the immune system can be augmented or manipulated to benefit improved health of animals are as follows.

The use of adjuvants to augment immune responses has been practiced for sometime. However, research on the mechanisms by which adjuvants produce their beneficial effects has been poorly understood until recently (Osebold, 1982). Adjuvants influence the degree of immunogenicity of antigens in vaccines by 1) serving as a repository of antigens where release into the body is slowed and by 2) selectively manipulating or favoring specific immune responses. Adjuvants such as Freund's incomplete or Freund's complete adjuvant or alumina gel have been used in the laboratory settings for years. Alumina gels have been incorporated into a number of commercial vaccines which are presently in use. Recently, refined adjuvants such as muramyl dipeptide, polyadenylic-polyuridylic acid complexes (poly A:U), Bordetella pertussis, saponin among others have been used in laboratories and on a limited commercial basis. The characterization of the immune responses associated with these adjuvants now makes it possible to manipulate the immune responses so that the most effective protective immunity can be attained with antigens. The use of these adjuvants will become more important with the increasing use of subunit or recombinant DNA products as well as with some of the current vaccine preparations.

Interluekins are hormone-like substances by which cells of the immune system communicate with each other. These substances, also known as interferon and interleukin II, are released from lymphocytes that have been activated by foreign antigens. This activated lymphocyte in turn releases the interleukins which alert and activate other lymphocytes. The activated lymphocytes are much more efficient and effective in controlling infections. Recombinant DNA technology has resulted in cloning the genes which code for the interleukins making them available for therapeutic use. There is now evidence that administration of these products to cattle and pigs can activate their immune systems. In some trials there is evidence of protective beneficial effects as well as an adjuvant effect when administered with vaccines.

Immunogenetics or identification of gene markers which are associated with characteristics of immune responses or host defense are being applied to food animals. Excellent examples of disease resistant strains of poultry to pullorum disease, Marek's disease and lymphoid leukosis have been described in chickens (Hutt, 1982). Evidence of resistance to internal parasites has been described in sheep and resistance to mastitis has been described between cows. Selection of disease resistant strains has been underway in chickens and plans are being made to undertake similar studies in cattle. By utilizing embryo transfer technology, reasonably rapid progress can be made in cattle as the markers for disease resistance traits are identified.

Monoclonal antibodies are now being used effectively in the treatment and temporary prevention of scours (diarrhea) in newborn calves (Sherman, 1985). These antibodies developed in mouse cell lines are directed to antigens on pili of _Escherichia coli_ bacteria which cause the diarrhea. The monoclonal antibodies cover the pili and prevent the E. coli bacteria from attaching to the lining of the intestines. As a consequence these bacteria are unable to cause disease. Other products of this nature are being developed which will have a major impact on treatment and prevention of animal diseases.

Monoclonal antibodies are also being used for diagnostic tests. These reagents are being developed and applied to a variety of infectious disease agents such as _Brucella sp_, _Pasteurella sp_, bluetongue virus, (Appleton 1983), _E. coli_ pilus and infectious bovine rhinotracheitis virus, among others. These antibodies are highly specific, they can be made in large quantities and consequently, they are easily standardized. Incorporation of these antibodies in ELISA test kits are being tested and in some cases they are now in commercial production. Many of these tests are rapid and accurate and they can be performed by veterinarians (Jacobsen, 1982). The recognition and confirmation of disease by veterinarians in a matter of minutes or hours will help in the control and treatment of these diseases.

III. Major Breakthroughs Needed

The major constraints which could prevent implementation of the breakthroughs addressed above include 1) research support for field testing, 2) commercialization of products and 3) development of standards and quality assurance of products. Research support is available for laboratory studies and limited animal trials. It is important that data management and systems analysis be done in the setting of large livestock operations. This setting provides the opportunity to address relevant problems and make adjustments appropriate to the system. Application of biotechnology to producer/owner livestock operations is best accomplished by researchers field testing products. The livestock producer can participate and observe the benefits of the applied technology. In many instances funding is not available for development and transfer of the technology to the producer.

There has been a reluctance on the part of some established animal health biological and pharmaceutical companies to accept and to incorporate the new biotechnology into their research and production programs. Reasons that some established companies have not involved themselves may be attributed to the costs of hiring new personnel, reequipping plants and financing the costs of product testing. On the other hand, the venture capital companies have undertaken the major responsibility for developing new products. Their major constraints include experience in understanding and testing products for use in animals and in the establishment of marketing systems that will be accepted by veterinarians and producers.

A major deterrant in getting products from the research laboratory to the livestock producer for use on his/her animals is with the quality assurance programs. Quality assurance of products is essential, however, efforts must be made to streamline the process and more economical ways of testing products must be devised. The establishment of general guidelines for product testing would help formalize the process. Some progress has recently been made in streamlining the process of developing guidelines and testing drugs, however, continuing efforts along these lines are needed.

IV. Institutional Arrangements

Research efforts are carried out in laboratories supported primarily by federal and state funds and by industry. Industry contributes about 3% to animal health research in universities (Osburn, 1984). Nearly all of the research that is being undertaken on data management and systems analysis is supported by State agricultural experiment stations.

The initial efforts for development of diagnostic technologies were made in universities through new funding for animal health and disease research and special grants programs. The USDA/ARS laboratories are now actively participating in this research. Recently a number of venture capital companies and a few established veterinary biologics laboratories have been acquiring diagnostic tests from university and federal researchers and they are incorporating these tests into their marketing programs.

Biotechnology relating to the development of genetically engineered products is being done primarily in public universities, state agricultural experiment stations, federal laboratories and by private industry. Presently the concentration of scientific expertise for genetically engineered products is found in venture capital companies. These companies have provided lucrative salaries and working conditions which have lured many researchers from universities. The fostering of collaborative efforts between all segments of the private and public research communities are showing promising returns.

Studies on disease resistance and immunogenetics are receiving increasing interest. Over the years most of this research has been conducted by State experiment stations. Recently, federal laboratories have initiated research in this area.

V. Likely Timing of Commercial Introduction

Commercial introduction of new technologies for disease control are beginning in a few instances. Examples of the application of computerized data base management systems for herd health have been started for a number of dairies and large swine confinement rearing operations in California, Michigan, Minnesota, New York, and England (Mather, 1983). In most cases, programs are still being developed and methods for analyzing and interpreting data are being undertaken. A number of computer programming companies are now involved in developing programs and some are for sale. As these systems become perfected, the interest and use will increase dramatically. Within 5 to 10 years programs for animal health will be widely accepted by progressive livestock producers.

Diagnostic technology will be in the hands of veterinarians and farmers the next 5 to 10 years. A number of venture capital firms are interested in marketing rapid diagnostic kits and systems. Tests for antibiotics in milk and diagnosing pregnancy in cattle will have widespread acceptance. The market place is in need of these systems and if economical tests can be developed, their use will be widespread and immediate. A few tests are now available for some animal problems.

Selection of animals for disease resistance is a relatively slow process and it will be 15 years before a significant impact can be realized in animal agriculture. The most rapid progress will be made in the poultry industry where the genetics, life span, and reproductive capacity exceed those of mammals. With the increasing interest and the application of newer technology such as gene insertion, nuclear transfer, and embryo transfer, some commercialization will be taking place in this area by 2000.

Genetic engineering of micro-organisms and embryos will evolve over the next fifteen years. The more promising area for the early commercialization will be with vaccines for viruses, with hormones and other products such as interferons and interleukins. Some products have been developed, however, they need to be tested for efficacy before they are commercially viable. Within 5 to 10 years subunit products such as the vaccine for foot and mouth disease virus made by recombinant DNA technology should be available for commercial use (Boothroyd, 1981). The commercialization of genetically engineered embryos will take at least fifteen years before it will be used.

In the area of immunobiology commercial products will continue to be introduced as they prove to be beneficial. New adjuvants will be incorporated into vaccines and the use of monoclonal antibodies for prevention and treatment of diseases will be introduced. Recently a monoclonal antibody for calf diarrhea was released by a company. As these products are shown to be effective on the farm their acceptance will be more widely embraced and more products will be introduced.

VI. Potential Primary Impacts

The major impact of the application of computer technology and biotechnology is to increase production efficiency. It is only through these methods that the cost of a farm operation can be minimized to keep the cost of food down. The data management and systems analysis are processes to assist veterinarians and livestock managers in being more efficient and for making sound management decisions. This becomes of increasing importance where large numbers of animals are being managed with a minimum of labor. Currently, record keeping systems are being used on dairies and in veterinary practices (Mather, 1983). Similarly the newer diagnostic technologies should expedite decision and curb the spread of disease. Both of the data management and diagnostic technologies should increase yield and reduce labor costs.

Genetic engineering for microorganisms and embryos and immunobiology are the application of new technology for preparation of vaccines, hormones and resistance factors. In some instances new products are being developed. The products should be safer and hopefully more effective in the prevention or treatment of costly diseases. Any reduction in the estimated 16 to 20% loss in total productivity through disease control can ultimately be passed on to the consumer.

Selection of animals for disease resistance is the ideal situation. This would reduce costs resulting from untimely deaths, labor involved in nursing and in disease prevention/treatment costs.

References

Ackermann, M., Peterhans, E., Wyler, R., "DNA of Bovine Herpes Virus Type 1 in the Trigeminal Ganglia of Latently Infected Calves", Am. J. Vet. Res. 43(1): 36-40, 1982.

Appleton, J. A. and Letchworth, G. J., "Monoclonal Antibody Analysis of Serotype-Restricted and Unrestricted Bluetongue Viral Antigenic Determinants," Virology 124:286-299, 1983.

Bolton, D.C., Chu, H.J., Ardans, A.A., et al, "Evaluation of the Critical Parameters of a Sensitive ELISA Test Using Purified Infectious Bovine Rhinotracheitis Virus Antigen", Veterinary Microbiology 6:265-279, 1981.

Boothroyd, J.C., Highfield, P.E., Cross, G.A.M., et al, "Molecular Cloning of Foot and Mouth Disease Virus Genome and Nucleotide Sequences in the Structural Protein Genes", Nature 290:30 April, 1981.

Ellens, D.J. and DeLeeuw, P.W., "Enzyme-Linked Immunosorbent Assay for Diagnosis of Rotavirus Infections in Calves", Am. J. Vet. Res. 42(12)2149-22150, 1981.

Farrell, C.J., Shen, d.T., Wescott, R.B., et al, "An Enzyme-Linked Immunosorbent Assay for Diagnosis of Fasciola hepatica Infection in Cattle", Am. J. Vet. Res. 42(2)237-240, 1981.

Hutt, F. B. and Rasmussen, B. A., Animal Genetics, (New York, NY: John Wiley and Sons, 1982).

Jacobsen, R. H. Downing, D. R. and Lynch, T. J., "Computer-assisted Enzyme Immunoassays and Simplified Immunofluorescence Assays: Applications for the Diagnostic Laboratory and the Veterinarians Office," J. Am. Vet. Med. Assn. 181(10):1166-1168, Nov. 15, 1982.

King, N. B., "Contributions and Needs of Animal Health and Disease Research", Am. J. Vet. Res. 42(7) 1093-1108, July 1981.

Mackett, M., Yilma, T., Rose, K. J. and Moss, B., "Vaccina Virus Recombinants: Expression of VSV Genes and Protective Immunization of Mice and Cattle", Science 227 433-435, 1985.

Mather, E., McPherson, M., Harsh, S., et al, "Food Animal Health Resource Management System (FAHRMX) - An Overview," Proc. Third International Symposium on Veterinary Epidemiology and Economics, (Edwardsville, Kansas: Veterinary Medicine Publishing Company, 1983).

McGuire, S. J. and Castro, A. E., "Evaluation of a Commerical Immunoassay for Rapid Detection of Rotavirus in Fecal Specimens from Domestic Species," 25th Annual Proc. Amer. Assn. Vet. Lab. Diag., 375-388, 1982.

Osburn, B.I., Bowen, J.M., Dierks, R.F., et al, Animal Health Research in American Agricultural Research (Morrilton, Arkansas: Winrock International Conference Center: The Association of American Veterinary Medical Colleges - Council of Deans), February 24, 1984.

Osebold, J. W., "Mechanism of Action by Immunologic Adjuvants," J. Am. Vet. Med. Assn. 181(10):983-987, Nov. 15, 1982.

Pilacinski, W. P., Glassman, D. L., Krzgyek, R. A., Sadowski, P. L. and Robbins, A. K., "Cloning and Expression in Escherichia coli of the Bovine Papillomavirus L1 and L2 Open Reading Frames". Bio/Technology, April, 1984.

Roy, P., Ritter, G. D., Akashi, H., Collisson, E., and Inaba, Y., "A Genetic Probe for Identifying Bluetongue Virus Infections in vivo and in vitro". *J. Gen. Virol*. 66:1613-1619, 1985.

Robbins, A. K., Weis, J. H., Enquist, L. W. and Watson, R. J., "Localization of a Pseudorabies Virus Glycoprotein Gene Using an *E. coli* Expression Plasmid Library", UCLA Symposium on Molecular and Cellular Biology, New Series, (New York, New York: Alan R. Liss, Herpesvirus, UCLA Symposium on Molecular Biology, New Series, Vol 21 ed Fred Rapp, 1984)

Sherman, D. M., Acres, S. D., Sadowski, R. L., Springer, J. A., Bray, B., Raybauld, T.J.G. and Muscoplat, C. C., "Protection of Calves Against Fatal Enteric Colibacillosis by Orally Administered *Escherichia coli* K-99-Specific monoclonal Antibody", *Inf. and Immun.* 42(2) 653-658, 1983.

Smith, B. P., Reina-Guerra, M., Hocsith, S. K. et al, "Aromatic-dependent *Salmonella typhimurium* as Modified Live Vaccines for Calves," *Am. J. Vet. Res.* 45(1)59-66, Jan. 1984.

Squire, K.R.E., Chuang, R. Y., Chuang, L. F., Doi, R. F. and Osburn, B. I., "Detecting Bluetongue Virus RNA in Cell Culture by Dot Hybridization with a Cloned Genetic Probe", *J. Virol. Methods* 10(1) 59-68, 1985.

Stott, J. L., Squire, K.R.E., Oberst, R., Cherrington, J. M. and Osburn, B. I., "Application of Modern Biotechnology to the Diagnosis of Bluetongue Virus Infection", *27th Annual Proc. Amer. Assn. Vet. Lab. Diag*. 35-50, 1984.

Werenne, J., "Interferon in Veterinary Medicine: The Present Position and Future Practice", Outlook on Agriculture, 14(3), 1985.

Background Paper No. 5

Animal Nutrition

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ANIMAL NUTRITION: EMERGING TECHNOLOGIES

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ANIMAL NUTRITION

Summary

The U.S. food animal industry is immense. Food animals provide 70 percent of the protein, 35 percent of the energy, 80 percent of the calcium, 60 percent of the phosphorus, and significant proportions of the vitamins and mineral elements in the average human diet in the United States.

The future of the food animal industry will depend not only on profitability, but also on the industry's adoption of new technology and on industry response to consumer concerns about cost, esthetics, convenience, and health. Areas of nutrition research that may result in major advances in animal food production and use in the next 20 years include (1) the relation of animal product consumption to human health, (2) alimentary tract microbiology and digestive physiology, (3) voluntary feed intake control, (4) maternal nutrition and progeny development, and (5) aquaculture.

Many consumers are concerned about the effect on human health of consuming animal food products, because of the amount of composition of fat in those products as well as the amount of sodium, nitrates, and potentially harmful bacteria or chemical residues. Studies have suggested strong links between some of these factors and human cancer, osteoporosis, and cardiovascular disease. Research on line is addressing these concerns by applying nutritional and genetic principles to the improvement of animal food products. For example, changes in animal fatty acid composition will be possible by using "protective" feed additives in specific animal diets. Changes in total animal fat content will probably occur through energy restriction, nutrient partitioning, and genetic selection. Sodium content of animal products can be reduced at the processing stage.

The direct impact of advances in this area will be animal food products that are safer for human health. The indirect impacts may be greater, however: To produce such products, producers may have to switch more to pasture, forage, and nonconventional feed resources. Such adjustments could change the total profile of agriculture.

Research into factors controlling voluntary feed intake and nutrient partitioning will result in the diversion of the use of nutrients from body maintenance to lean tissue growth and other productive functions. Such methods will save feed and provide opportunities for alternative uses of feed resources.

More complete knowledge of maternal nutrition in relation to fetal survival and prenatal and postnatal development may lead to significant increases in the amount of edible product per breeding unit. This outcome will be translated into savings in labor and resource use.

Finally, aquaculture has emerged as an important new field of animal agriculture in the United States. Research into specific nutrient requirements for different species of fish during all phases of the life cycle and interactions between nutritional requirements and water environment will provide new technology that will allow the industry to continue to improve its competitive position in animal agriculture. Future growth of private aquaculture will provide an additional supply of edible fish and shellfish for consumption by the U.S. population, whose per capita appetite for animal products may be saturated.

Animal Nutrition: Emerging Technologies

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ANIMAL NUTRITION: EMERGING TECHNOLOGIES

Introduction

The food animal industry in the U.S. is immense; the daily *per capita* consumption of meat and animal products has been about 1200 to 1400 calories for the past two decades. Foods of animal origin provide 70 percent of the protein, 35 percent of the energy, 80 percent of the calcium, 60 percent of the phosphorus, and significant proportions of the vitamins and mineral elements consumed in the average diet in the U.S. Trends in *per capita* consumption of animal products suggest that we probably have reached the saturation point in the United States. *Per capita* consumption of chicken and turkey meat in the U.S. has increased 70 percent since 1963 (17.0 kg in 1963 and 28.9 kg in 1982). Pork consumption has maintained a relatively flat curve, averaging 27.4 kg *per capita* yearly, while beef consumption increased steadily to 43.4 kg in 1976 and then declined to 35.1 kg in 1982, a level of about 12 percent above that in 1963. Veal consumption has declined 60 percent to 0.73 kg and lamb and mutton 66 percent to 0.68 kg while fish consumption has increased slightly during the period from 1963 to 1983, although it still represents less than three percent of total animal product consumption in the U.S. The longterm future of food animal production in the U.S. will depend not only on the ability to produce meat and other animal products at a profit, but on the adoption of new technology by the animal industries and on their adaptability to the esthetic, convenience, health and economic concerns of the consumer. This adaptability will depend on efficient resource utilization which, in turn, depends on complex interactions among all segments of agricultural research, production and marketing.

Animal nutrition research must combine traditional methods with modern multidisciplinary approaches. New biotechnology (U.S. Congress, OTA 1984) offers the promise of providing the basis for commercial large scale production of hormones, enzymes, amino acids, and other metabolites (e.g., growth hormone, cellulase, tryptophan and threonine, endorphins) which may be applied in animal nutrition. Greater alignment of animal scientists with molecular biologists and biochemists will help assure that the opportunities for breakthroughs in food animal production brought about by commercial efforts in new biotechnology will flourish.

Major Lines of Research From Which Significant Technologies are Likely to Emerge

Animal nutrition research currently underway in universities, government and private industry addresses a broad array of basic and applied problems. Specific lines of research which appear to offer a high probability of yielding major advances of significance in animal food production and consumption and human health in the next 20 years are as follows:

Animal Product Consumption and Human Health
Alimentary Tract Microbiology and Digestive Physiology
Voluntary Feed Intake and Efficiency of Animal Production

Maternal Nutrition and Progeny Development

Aquaculture

Animal Products Consumption and Human Health

Although foods of animal origin are recognized as important sources of many required nutrients (National Research Council, 1976, 1978, 1980b; Oltjen and Dinius, 1975; CAST, 1980), there is public concern about the effect of animal fats and animal proteins and of tissue residues of chemicals administered to animals and animal products on human health. Relationships between diet and severe chronic degenerative diseases, including atherosclerotic heart disease, hypertension, cancer and osteoporosis have been postulated (Harper et al., 1980; NRC, 1980b, National Center for Health Statistics, 1979a, 1979b, 1980). The specific issues relating diet to disease which impinge on the future of the food animal industry focus on: type and quantity of fat and amount of cholesterol in the diet in relation to the high incidences of cardiovascular disease and cancer in the U.S.; consumption of high fat-low plant fiber diets in relation to obesity and certain types of cancer (i.e., colon); calcium, phosphorus and protein contributions to the diet by animal products in relation to long term losses of bone calcium (i.e., osteoporosis and bone fractures). Reports issued by several recent expert panels have suggested the possibility of links between consumption of animal products and the high incidence of these diseases in the U.S. population (National Research Council, 1980b, 1981b, 1982). These panel reports have been based on the results of epidemiological studies, animal experiments and clinical intervention experiments.

A growing number of consumers perceives that animal product consumption at the current level in the U.S. may be harmful. This perception has been reinforced by the recent clinical study showing a significant decrease in heart disease among a selected population of males with familial hypercholesterolemia whose serum cholesterol levels were lowered by dietary means or drug therapy (cholestyramine ingestion; Kolata, 1984). Other important issues are: sodium content of processed foods (i.e., ham, sausage, cheese) in relation to development and treatment of hypertension; nitrate addition to cured meat products in relation to cancer; the threat of harmful residues of chemicals or their metabolites remaining in edible products of animals administered xenobiotics; the possibility of human health hazards associated with antibiotic resistant strains of pathogenic bacteria as a result of wide use of antibiotics in animal feeds (Visek, 1978; CAST, 1980, 1981). The documented and generally acknowledged nutritional contributions of animal products in the human diet are often ignored or undervalued by those expressing these issues and concerns. None of these issues have been resolved.

In biological systems, the ultimate answers must be obtained in the target species, in this case, the human. Thus, clinical studies now underway must continue; however, such studies often cannot address fundamental questions of cause and effect and must be accompanied by animal experiments whose results can then be applied to studies in humans.

Current research aimed at establishing the relationship between animal product consumption and human health falls into two broad categories; research in each category has a high probability of yielding useful information.

Animal Models for Human Nutrition. Research underway at several private and public laboratories has shown that atherosclerosis can be induced by dietary means in some genetic strains of swine (Pond, 1982). Because of the similarity of swine and humans in many anatomical and physiological aspects, the information gained with swine can be extrapolated with reserved confidence to humans. Evidence with miniature swine and some strains of conventional swine indicates that diets containing 10 to 20 percent saturated fats and up to one percent cholesterol produce hypercholesterolemia and atherosclerosis within months; other dietary variables, including carbohydrate and protein source, protein:calorie ratio, levels of certain vitamins and minerals and the presence of oxidized cholesterol are under continued scrutiny in cardiovascular research in swine (Stanton and Mersmann, 1985). The pig is a particularly useful model for human nutrition research because of its similarity to humans in cardiovascular and gastrointestinal anatomy and physiology. In addition to the pig, poultry, cattle and other ruminants are used in increasingly large numbers for aspects of nutrition related to human health (Institute of Laboratory Animal Facilities and Resources, 1980). These efforts are essential components of the total research thrust directed toward human health. Data obtained from animal experimentation cannot be replaced by computer modeling and simulation techniques.

Clinical Experiments in Humans Consuming Animal Products.

Results obtained from clinical nutrition studies with animal products carry with them the implication that animal product composition is constant and refractory to change. This is not true. It is generally recognized that fat content of most animal products is higher than that of most plant foods. However, the percentage of fat in meat can be reduced by genetic selection or by feeding programs designed for minimizing fat deposition (Figure 1 for pork) or by physical removal of the external fat during processing (an inefficient approach). Furthermore, composition of the fat can be changed by dietary means; chicken fat and pork fat resemble the composition of the fat in the diet, while in ruminants, whose rumen microbes hydrogenate unsaturated fat and produce large amounts of short chain fatty acids by fermentative processes, changes are more difficult. The fat composition of ruminants can be altered experimentally by appropriate feed processing techniques which result in rumen by-pass of ingested unsaturated fats, a high proportion of which are deposited to yield tallow or lamb fat more unsaturated than conventional fat from ruminants. Research into the alteration of body lipid composition by dietary manipulation in ruminants offers promise of major changes in fatty acid composition of beef and lamb. Such manipulation with "protective" feed additives and

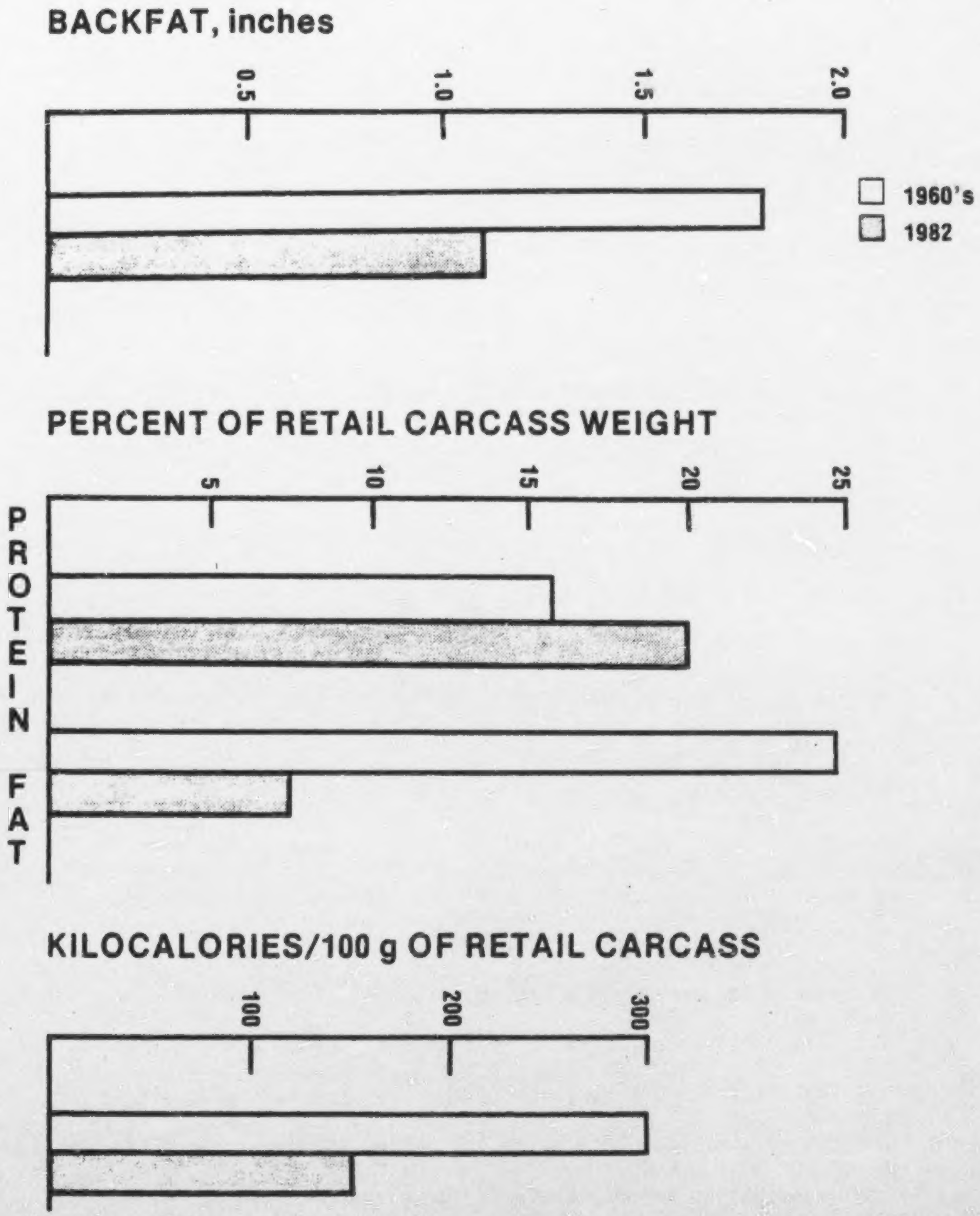

Figure 1. Backfat thickness, percentage of protein and fat in retail pork carcasses and kilocalories of energy per gram of edible pork carcass in 1960's compared with 1982.

appropriate diet composition could promote a major shift in the perception of beef and lamb by consumers concerned with potential health hazards of animal products. Similar shifts in fat content and composition of milk are feasible under experimental conditions in the dairy industry, but incentive for producers to apply this technology is impeded by current pricing structures in which whole milk from the farm is price discounted if fat content falls below 3.5 percent.

The cholesterol content of beef, lamb and milk fat is no higher than that of animal products such as poultry and fish, but blood plasma cholesterol of humans consuming the former group is sometimes greater than that of humans consuming poultry and fish. This is because beef, lamb and milk contain a higher level of saturated fats and this is associated with an increase in cholesterol absorption from the intestinal tract. Cholesterol concentration of animal fat appears to be relatively refractory to change by dietary (Bitman, 1976) or genetic means. Therefore, changes in fatty acid composition of animal fats by dietary manipulation and in total fat content by energy restriction, and/or genetic selection appear, to offer the highest probability of success in meeting emerging consumer demands for lean meat of acceptable fat content and composition.

The relationship between dietary salt intake and hypertension is clear for a proportion of the U.S. population unable to dispose of excessive sodium via the kidney. Average per capita salt consumption is well above the level recommended by the

National Research Council (1980a). The Food and Nutrition Board has recommended that salt consumption be reduced from the present 10 to 12 gram daily to 3 to 8 gram (Pearson and Wolzak, 1982). This would require a reduction in the amount of salt added to processed animal products in addition to a restriction in discretionary use of salt as a condiment by consumers. Research underway to establish more clearly the mechanism of high salt intake in producing hypertension and to study sodium substitutes will provide important information on how processed animal products can be produced in a way more conducive to human cardiovascular health.

The potential threat of antibiotics, toxins, chemicals and microbial contaminants in animal feeds to human health (CAST, 1980, 1981, 1982; National Research Council, 1981b, 1982) needs continued research and surveillance. The widespread use of antibiotics as growth stimulants in animal feed has raised concern about the potential for human health hazards related to development of antibiotic resistance by pathogenic microorganisms in humans (CAST, 1981).

The information urgently needed to clarify the role of high fat-low fiber diets in obesity and colon cancer, the role of calcium, phosphorus and protein intakes on bone integrity and the magnitude of the risk of harmful tissue residues in the meat and milk of animals administered xenobiotics will be derived from research on animal models as well as from continued clinical trials in humans.

The future of the food animal industry in the U.S. will depend, to a major degree, on the perception by the public of the healthfulness and safety of animal products in the diet. The ability of the animal industry to respond favorably to these perceptions will be influenced by the rate and extent to which the unanswered questions addressed above can be resolved. Research in animal nutrition must have a multidisciplinary approach wherein such fields as physiology, biochemistry, genetics, microbiology and biotechnology play a major role. Evidence now emerging from current and recent research suggests that the present human health concerns related to animal product consumption can be addressed by appropriate manipulations of meat and milk composition through application of nutritional and genetic principles by the animal industry. However, such changes will require economic as well as attitudinal adaptations by livestock producers, processors, distributors and consumers.

Alimentary Tract Microbiology and Digestive Physiology

A large body of knowledge exists concerning the rumen microflora and microfloral-host interactions in ruminant animal nutrition. However, there is a dearth of information available on the role of microflora in the lower gastrointestinal tract of ruminant and nonruminant animals (Oltjen et al., 1980).

Microbes (bacteria, protozoa, fungi) in the rumen utilize fibrous feedstuffs consumed by the host. Their cell mass and the fermentation products are digested and absorbed and represent a major portion of the total protein and energy required by the host. Microbes interact to improve the digestion of complex carbohydrates such as cellulose, they provide nutrients required for the growth of other microbes, and produce volatile fatty acids, vitamins and amino acids used by the host. The highly complex metabolic activities and interactions underway in the rumen have been studied effectively by modeling techniques. Such techniques have allowed the evaluation of current concepts of ruminant digestion, enhanced the number of experimental measurements possible and provided a basis for studying several aspects of rumen function simultaneously. Modeling techniques are currently under study to address factors involved in particle size reduction, regulation of water dynamics, dynamics of protein degradation and nitrogen metabolism and factors affecting microbial growth (Baldwin and Allison, 1983; Owens and Bergen, 1983).

Information on the utilization of dietary fiber for energy by nonruminants is limited. There is evidence for individual variation among humans and swine (Gargallo and Zimmerman, 1981; Varel et al., 1982). These differences, whether environmental or genetic, are associated with the ability to harbor active cellulolytic bacteria. Microbes in the cecum and colon ferment significant amounts of cellulose and other cell wall materials. Kass et al. (1980) estimated that up to 14 percent of the energy requirements for maintenance could be derived from fermentation of cellulosic substances in the lower intestine of growing swine; in adult swine the value may be considerably higher. The gastrointestinal tract weight is increased in some strains of pigs fed high fiber diets, the ability of the animal to utilize fiber (i.e., increase cellulolytic bacteria) appears to be associated with the gastrointestinal tract weight response, but the mechanism is unknown.

The upper and lower intestinal tracts of ruminants and nonruminants respond to substances ingested and integrate such physiological processes as motility of the intestine, blood flow, and release of enzymes, hormones and other secretions needed for optimum digestion and absorption of nutrients.

Chemical methods of determining composition of plant cell walls do not predict accurately their digestibility by the host animal or the anaerobes inhabiting the gastrointestinal tract. Lignin chemistry and lignocellulose bonds are under study, but further work is needed to understand the breakdown processes sufficiently to allow the separation of lignin from cellulose to increase the amounts of carbohydrates utilized by the animal. Gene splicing techniques to clone cellulase genes from microorganisms (Montencourt, 1983) may be applied soon in nutrition by changing the microbial activities in the gastrointestinal tract of animals to enhance the capability of breaking the holocellulose-lignin bond and to increase the production of cellulase or, perhaps before that, by providing the basis for production in mono-culture of superior cellulytic microbes which degrade cellulosic feedstuffs to more readily available energy forms for use in animal feeding. Such developments will drastically change the constraints on both ruminant and nonruminant production imposed by inadequate capacity of the digestive system to assimilate large amounts of lignocellulosic feedstuffs. The use of chemical fractionation procedures, infrared reflectance analyses, transmission and scanning electron microscopy techniques, nylon bag digestion methods and rate of passage modeling procedures have revealed important variations in the nature and ultrastructure of plant cells that influence extent and rate of digestion of plant fibers *in vivo*.

The methods of harvesting, storage and processing of fibrous plant materials affect not only utilization, but the availability of nitrogen from the plant. Methods for estimating rumen and postruminal protein synthesis, degree of solubility or degradation of protein in the rumen along with techniques for estimating reduced nitrogen availability due to heat damage of hays and silages have been developed recently (Reid and Klopfenstein, 1983). Nitrogen in feeds can be partitioned into soluble and insoluble fractions; the concept of ruminal bypass protein has evolved, which opens the possibilities for improving the efficiency of conversion of dietary proteins to animal products in ruminants. Useful models for examining utilization of fibrous nitrogenous components of the ruminant diet are in use in research. Significant advances have been made in estimating feed intake and utilization by grazing animals using surgically prepared rumen, esophogeal and duodenal or ileal fistulas to accommodate application of indigestible indicator techniques (i.e, rare earths, chromium oxide) and compartmental modeling equations for describing ingesta passage.

Although relatively low prices of grains and concentrate feeds have limited the expanded utilization of forage resources in the U.S. during the past 25 years, changing economic conditions associated with higher grain and concentrate prices portend greater dependence on forages for livestock feeding in the future (Reid and Klopfenstein, 1983). Pastures and forages indigenous to specific regions of the U.S. and crop residues (more than 400 million metric tons/year in the U.S., Larson, 1979) constitute an enormous feed resource to support the livestock industry of the United States. The rate and extent to which this resource will be utilized for animal production over the next decades will be greatly affected by the progress of current research on chemical and physical treatment of forages and on alimentary tract microbiology, and digestive physiology, some aspects of which have been addressed briefly in this section.

Voluntary Feed Intake, Nutrient Partitioning and Efficiency of Animal Production

It is generally accepted that maximizing feed intake of growing and lactating animals is associated with improved efficiency of production (edible tissues or milk). However, information on nutritional, environmental and metabolic mechanisms to increase or decrease voluntary feed intake and control nutrient partitioning into lean and fat tissues of food-producing animals in different productive states is inadequate. Ingestive behavior is a consequence of changes in the body associated with central nervous system responses. Such stimuli as rumen fill, acetic acid concentration in the rumen, skin temperature and circulating levels of hormones such as cholecystokinin and estrogen affect the onset and ending of feed ingestion (Curtis and Houpt, 1983). Metabolizable energy (ME) consumed during growth is partitioned between heat production (HP) and body tissue gains (BTG)(mainly fat and protein). As mature size is approached, the proportion of ME directed to BTG declines. In the mature, nonpregnant, nonlactating animal at rest and in steady state, ME consumed = HP. An increase in feed intake above theoretical maintenance level in the growing or lactating animal should result in more efficient conversion of feed nutrients into BTG or milk because a higher proportion of ME consumed will be directed to these productive functions. Assuming that the digestive system has the capacity to utilize incremental increases in nutrient intake, any extrinsic or intrinsic stimulus to increase feed intake should result in greater biological efficiency of nutrient utilization once maintenance requirements have been met. In economic savings, in the case of growing animals, this occurs via shortened time to market. Newly developed electronic monitoring devises to record feed intake and eating patterns of individual animals offer the potential to enhance the understanding of feed ingestion patterns.

The endocrine control of digestive function and long and short term feed intake of food animals is poorly understood. Two major systems of control of feeding appear to be operative

(Baile, 1979). One has a primary function of maintaining a balance between energy demands and intake over long time periods (i.e., weeks, months); this system modulates the second system which controls meal size and frequency over short time periods (i.e., hours, days). Mature animals in steady state maintain a relatively constant body weight and energy content. Growing, pregnant and lactating animals also adjust feed intake to maintain a continuous nutrient supply to sustain continued growth or other anabolic function. In ruminants, volatile fatty acids from microbial fermentation are major energy sources and exert a control over feeding behavior by acting on peripheral or central nervous system tissues. In nonruminants, certain metabolites and hormones affect neural activity of the hypothalamus and other central nervous system structures. The role of peptide hormones of the gastrointestinal tract (i.e., cholecystokinin, secretin) and central nervous system (i.e., endorphins and cholecystokinin and its metabolites) in short term control of feed intake is an active area of current research both in ruminants and nonruminants. Recent advances in recombinant DNA techniques (U.S. Congress, OTA, 1984) have made available microbially produced hormones and other metabolites (e.g., microbially produced human growth hormone which has been shown to stimulate growth in pigs, Baile et al., 1983) which may be applied in efforts to understand and control voluntary feed intake.

Concepts are developing in nutrient partitioning as related to size and metabolic activity of visceral organs in response to diet composition and feeding level (Webster, 1980). Nutritional plane has a profound effect on visceral organ mass and, in turn, on fasting heat production (Koong et al., 1982). Current evidence suggests that the overall efficiency of nutrient utilization for growth of animals is closely tied to the size and metabolic activity of the gastrointestinal tract. Greater attention to this organ system and to genetic and environmental factors which affect it is now occurring in research.

Recent data (Baker et al., 1984; Ricks et al., 1984) illustrate the effectiveness of orally administered synthetic beta-adrenergic agonists in repartitioning of nutrients in growing animals to favor lean and reduce fat deposition. The use of this class of compounds (catecholamine derivatives) offers exciting possibilities for increasing lean meat production without increasing total feed requirement. Exogenous growth hormone administration increases lean tissue deposition in growing animals (Machlin, 1972; Baile et al., 1983; Chung et al., 1985) and improves milk production in cattle (Bauman and Elliot, 1983). The effect of these metabolic alterations on dietary nutrient requirements of animals of varying genetic background is largely unknown. The application of these potentially useful tools in animal production awaits the development of acceptable delivery systems.

Maternal Nutrition and Progeny Development

Nutrition and Reproduction. Reproductive efficiency, as measured by fecundity and prolificacy, is hindered by large prenatal and early postnatal losses, a high proportion of which are related to nutrition. For example, in swine, a 30 to 40 percent embryonic and fetal death loss is accepted as the norm (Cartwright et al., 1980). Early postnatal death may account for an additional 10 to 30 percent. These losses are multifactorial, but direct and indirect participation of improper nutrition is significant. In dairy cattle and beef cattle, delayed estrus following parturition (13-14 mo. calving interval) and low conception rates (70 to 75 percent) reduce total reproductive rates significantly. The effects of nutrition of beef cattle during the postpartum period on reproduction are profound (Dzuik and Bellows, 1983). There is evidence for endocrine effects related to a low plane of nutrition as causative factors in low reproductive performance in beef cattle (Bellows et al., 1979; Echternkamp et al., 1982). Availability of radioimmunoassay techniques for monitoring circulating levels of hormones has provided a powerful tool for studying the role of nutrition during the prepubertal, reproductive and lactation periods on the endocrine system of ruminants and nonruminants. More complete understanding of the physiological processes related to low reproduction in response to nutritional plane and the relative importance of specific nutrient intakes should be forthcoming as a result of current and future research with each food animal species. This understanding will be derived from long term studies over multiple reproductive cycles in which multidisciplinary approaches are used. Emerging technologies in improving reproductive efficiency in cattle and sheep by superovulation, embryo transfer and genetic selection for multiple births (e.g., Booroola Merino sheep), create the need for reassessing nutrient requirements for pregnant and lactating females. Long term studies will be needed to quantify nutrient requirements of cattle and sheep during various stages of the life cycle to accommodate the higher reproductive potential brought about by these new developments.

Nutrition and Progeny Development. Most of the current knowledge on the effect of the nutrition of the dam during pregnancy on the long term growth and development of surviving progeny was obtained from swine and laboratory animals. Severe feed restriction of swine during gestation reduces piglet birth weight, but has only minor effects on subsequent weight gain. Severe protein restriction causes a similar reduction in birth weight, but produces an apparently permanent stunting of weight gain (Atinmo et al., 1974). Progeny of dams fed one-third of the recommended daily feed allowance during the first two trimesters of pregnancy are leaner and have smaller fat cells at slaughter than progeny of control dams. In contrast to a report in humans (Ravelli et al., 1976) in which obesity was reported in young adult males whose mothers were subjected to food shortages in pregnancy, there is no evidence for hyperphagia or obesity in pigs or rats (Pond et al., 1985) from dams underfed during gestation. Cattle and sheep are subject to wide variations in available feed supply during certain seasons of the year under pasture and range conditions. The resulting fluctuations in nutritional status during different stages of pregnancy probably have unmeasured effects on the fetus which are manifested in postnatal life. Although such effects are virtually unmeasured in ruminants, data from other species suggest that important effects are likely to exist. Modern techniques of endocrinology, cellular metabolism and growth assessment encourage the quantification of maternal nutrition effects on progeny development in ruminants. Information gained from food animal species will have direct implications for humans, in which very limited information is available on the relationship of prenatal and perinatal nutrition to later growth, development and longevity.

Aquaculture

During the past decade a new field of animal agriculture, aquaculture has emerged. Total U.S. harvest of edible fish and shellfish was 1.5 million metric tons in 1982 (5.6 million metric tons total world production in 1978, Ryther, 1981) of which 11 percent (179,500 metric tons, Table 1) was produced by aquaculture (Natl. Aquaculture Develop. Plan, 1983). Private aquaculture produces 40 percent of U.S. oysters, most catfish and crayfish, and nearly all of the trout in the U.S. In addition to private aquaculture, federal and state governments produce fingerlings for stocking lakes and streams which provide benefits for both commercial and recreational fishermen (National Aquaculture Develop. Plan, 1983). Catfish, crayfish and trout

Table 1. U.S. Private Aquaculture Production[1] for 1982 (Estimate)

Species group	Value (1000 dollars)	Metric tons	Percent of total
Buitfish	100,000	15,000	8.4
Catfish	120,000	90,909	50.7
Clams	12,000	2,045	1.1
Crayfish	27,000	25,000	13.9
Freshwater prawns	1,800	182	0.1
Mussels	1,600	773	0.4
Oysters	34,000	9,878	5.5
Pacific salmon[2]	4,000	11,587	6.5
Tropical fish	20,000	-	-
Trout	48,000	28,818	12.2
Other species[3]	5,000	2,273	1.2
	373,400	179,463	100.0

[1] Live weight harvest for consumption, except for oysters, clams and mussels, which are meat weight. Meat produced from cattle, swine and sheep slaughtered in the U.S. in 1983 totalled 177 million tons.

[2] Pen-rearing only; ocean ranching returns are currently used for broadstock.

[3] Includes species such as sturgeon, paddlefish, carp, buffalo, tilapea, mullet, abolone, etc.

constitute more than one-half of the total U.S. private aquaculture production; catfish and crayfish production is concentrated in 10 south and southeastern states, while trout production is greatest in Idaho (Aquaculture Outlook & Situation, 1982).

Current knowledge of nutritional requirements of cold water and warm water fish is summarized in National Research Council publications (National Research Council, 1977, 1978a, 1981a, 1983). Continued refinements in specific nutrient requirements for different species of fish during various phases of the life cycle will be made through public and privately supported research programs in the United States. Nutrient requirements of fishes are affected by species, genetic strain, sex and sexual maturity as well as by such factors as body composition; energy density of the diet; bioavailability of the nutrients in the diet; nutrient interactions; water temperatures, flow rate and chemical composition; the presence of infectious diseases, toxins, enzyme inhibitors and other detriments in the environment (National Research Council, 1981a). Research on nutrient interactions and on interactions between nutrition and environment for each commercially important warm and cold water species will provide the kind of information needed for developing technologies in aquaculture that will allow the industry to continue to improve its competitive position with other segments of animal agriculture in the United States. In a recent report (National Research Council, 1978b; Production Credit Association, 1983), five potential programs were recommended in aquaculture which are not yet utilized on a commercial scale in the United States: cultivation of aquatic plants for production of feed and chemical products and for wastewater treatment; closed-cycle aquaculture; polyculture; ocean ranching; alternatives to current feeding practices (herbivorous rather than carnivorous species). Although scientific and technological obstacles remain, economic, political and administrative constraints have been judged as primary deterrents to the rapid expansion of aquaculture production (National Aquaculture Develop. Plan, 1983).

Future growth of private aquaculture in the U.S. will provide an additional supply of animal products for consumption by a U.S. population whose appetite for animal products appears to be saturated. Such competition will heighten the need for increasing efficiency of production by all segments of the food animal industry.

Major Breakthroughs Needed for Successful Completion of Each Technology

Animal Product Consumption and Human Health

Foods of animal origin are accepted as important sources of many nutrients not present in sufficient quantities in most plant sources. Yet, the growing perception that consumption of animal products may be associated with negative effects on health must be addressed squarely and definitive knowledge obtained. The extent of relationships between diet and severe chronic degenerative diseases (heart disease, cancer, osteoporosis) must be determined by a combination of clinical studies in humans and experimental animal model research. Concurrent with these studies must be research directed toward genetic, nutritional and processing procedures for altering nutrient (particularly fat) content and composition of animal products. The major constraints to progress in achieving those technologies are: resistance among animal scientists to the concept of a possible detrimental effect of animal products on human health and an associated reluctance to address the question seriously and; insufficient coordination of appropriate research efforts between medical and agricultural scientists. Additional funding of several millions of dollars annually will be needed for clinical and basic research, both in medicine and in animal science, to achieve the needed breakthroughs.

Alimentary Tract Microbiology and Digestive Physiology

Manipulation of alimentary tract microbial systems clearly affects animal performance. Optimum manipulation will require greater indepth knowledge of microbial metabolism and physiological parameters in the digestive tract of the host. Advancement of knowledge is hampered by incomplete understanding of the microbe-host interactions and their significance. Although dietary antibiotics have been used for more than 30 years to promote weight gain and improve efficiency of feed utilization in food animals, the mode of action of this significant response is still unknown. Systems of feeding or feed treatment that alter or by-pass rumen fermentation and accommodate progress in changing composition of animal fat and protein must be developed from multidisciplinary research. Application of gene splicing technology to unlock cellulose from lignocellulosic feedstuffs by ruminants and nonruminants will have profound effects on animal feeding practice as well as on plant science and economics. Rapid advances in integration of isolated knowledge of individual components of digestive physiology (i.e., microbiology vs animal digestive physiology and anatomy) must occur by a systems approach whereby scientists from several disciplines join in a common research effort. Most research laboratories in animal nutrition are not traditionally organized along such interdisciplinary concepts. This represents an overriding constraint. Additional funding for this type of research approach is important, but the funding must be directed partly toward training programs for young scientists. Very few gastrointestinal microbiologists and gastrointestinal physiologists currently are being trained in departments of animal science. Greater effort must be made to bolster training programs in these disciplines in animal science departments or develop better communication and coordination with medical science training institutions.

Voluntary Feed Intake, Nutrient Partitioning and Efficiency of Animal Production

Fundamental discoveries related to the neuroendocrine and other controlling factors in short and long term feed consumption are needed to allow the development of feeding strategies to maximize efficiency of feed utilization. With an improved understanding of the neural, humoral and molecular bases of short term appetite it should be possible to manipulate long term energy balance to fit production incentives, i.e., to stimulate feed intake and body nutrient accretion in growing and lactating animals and to inhibit feed intake in animals in which overfattening is to be avoided. Such control might be forthcoming from gene splicing technology to produce specific hormones, enzymes or other metabolites to be administered exogenously for specific purposes at prescribed stages of the life cycle of food animals. Other research must be directed toward a further understanding of the fundamental metabolic bases for the observed changes induced by beta-adrenergic agonists in repartitioning of nutrients for increased lean tissue deposition and the effect of such changes in body composition on dietary nutrient requirements. The basis for this work may be done with laboratory animal models and the developmental work with the target farm animal species. Funding for expanded research in this area must be directed first toward identification of specific substances controlling appetite (i.e., cholecystokinin), then toward application of recombinant DNA techniques for mass production of such substances and finally toward appropriate delivery systems for application in each animal species. Several million dollars annually should be directed toward completion of this research, the funding to be allocated by competitive grants.

Maternal Nutrition and Progeny Development

Endocrine x nutrition interactions in dam and fetus and a more fundamental understanding of uteroplacental physiology and endocrinology are needed to develop management strategies to minimize prenatal deaths and maximize efficiency of pre- and postnatal growth and development. A major impediment to improved maternal nutrition in beef cattle, sheep and swine appears to be a lack of understanding of interrelationships among such factors as nutritional status, climatic environment, endocrine function, specific infections, immunology, diseases, and genetic background. The large death losses observed prenatally and postnatally in swine are considered to be from multiple causes, yet the physiological processes associated with these large losses are poorly defined. Interdisciplinary effort will be needed to unravel the factors responsible for this major source of biological inefficiency. The Integrated Reproductive Management (IRM) program instituted in USDA and at state university research and extension centers should enhance the probability of making major progress through the combined efforts of research and extension personnel.

The effects of maternal nutrition on long term growth, development and longevity of surviving progeny are almost unknown. Breakthroughs in technology which will allow advances in efficiency of animal production by this route will rely on long term, generation studies with multidisciplinary inputs. This virtually untouched phase of animal production research needs additional sustained funding. This extended research support should result in the development of life cycle feeding systems for maximum resource conversion efficiency in animal production with spinoffs in biological principles for application in human nutrition (e.g., what is optimum maternal nutrition in relation to maximum longevity and health of the children?).

Aquaculture

Major issues in aquaculture research involve the high cost of closed-cycle aquaculture systems which require maintenance of life support systems and utilization of expensive feeds high in animal protein. Fish culture has not kept up with other animal agriculture, primarily because of limited definitive work on nutritional and husbandry requirements and barriers associated with marketing policies and procedures. Increased funding for research in all phases of fish production is needed to allow sustained growth of the industry in the United States. Such funding should be concentrated in existing federal and state research laboratories where expertise and resources are already available.

Institutional Arrangements

All five of the above research areas will require additional funding to be effective. The funding must come in two approaches: (1) Training of young animal scientists in basic sciences relevant to pursuit of these research areas. Currently, there is no structure at hand to provide for this need. Intrinsic to this approach is the need for aggressive pursuit of multidisciplinary collaboration among academic and research units representing animal production and molecular biology, including biotechnology. Such collaboration will enhance the opportunities for breakthroughs in food animal production. Training grant programs or similar organized efforts are urgently needed.

(2) Increased funding at government and university laboratories or in partnership research efforts between government, universities and private sector will be necessary to achieve progress in each area.

Likely Timing of Commercial Introduction of Each Technology

The application of research knowledge related to effect of animal product composition (amount and composition of animal fat) on human health can begin immediately for pork production, with refinements to occur continuously for pork for the foreseeable future. The knowledge that pork fat composition resembles diet fat composition and that lean pork can be produced by altering feeding procedures to produce lean pork high in polyunsaturated fatty acids gives the swine industry a headstart in responding to clinical research results linking the ingestion of large amounts of animal fat with serum cholesterol and heart disease. Technology transfer of this type in production of beef, lamb and milk will be slower due to the lagging body of knowledge related to dietary manipulations and body and milk composition.

The time anticipated before commercial application of technologies derived from additional research in alimentary tract microbiology and digestive physiology; feed intake control; maternal nutrition and progeny development; and aquaculture will vary from a minimum of five years to up to 10 to 20 years for technologies such as those of commercial aquaculture in which an array of nutrition and production strategies are evolving simultaneously. The high risk areas of research addressed here are fraught with uncertain outcomes and the full impact of research in some technologies will not be assessed adequately until early in the 21st century.

Potential Primary Impacts of Each Technology

Animal Product Consumption and Human Nutrition

The direct impact will be largely in terms of producing animal products in conscious response to consumer needs. The indirect impact may be greater, because feeding strategies may change to dependence on greater amounts of pasture and forage crops and on utilization of nonconventional feed resources. Such adjustments will affect agronomy, plant breeding, and marketing, and may change the total profile of agriculture.

Alimentary Tract Microbiology and Digestive Physiology

Breaking the holocellulose-lignin bond and unlocking cellulose by cellulase producing microbes will create revolutionary changes in feeding practices such that plants high in lignocellulose can be used at higher concentrations in diets for animals. This change in feeding practices will be associated with changes in cropping needs, feed storage and processing strategies and in competition between animals and humans for food and land resources.

Voluntary Feed Intake, Nutrient Partitioning and Efficiency of Animal Production

Diversion of nutrients to lean growth and other productive functions rather than to use for maintenance functions by controlling voluntary feed intake and the use of nutrient repartitioning agents will create savings in feed resource requirements and provide opportunities for alternative uses of feed and feed resources. Increasing feed intake will make more nutrients available for growth once maintenance requirements have been met.

Maternal Nutrition and Progeny Development

An increase of 20 percent in overall biologic conversion rate (edible product per breeding unit) seems to be a realistic possible attainment. If this figure is equalled or exceeded, the improvement will be translated in terms of labor saving as well as resource utilization.

Aquaculture

As the commercial production of fish grows in the U.S. it will create need for adjustments in production of other animal products in response to changing supply and demand of animal proteins in a society apparently saturated in its *per capita* demand for animal products in the diet.

Although the focus of this discussion has been on the United States, the impact of each emerging technology obviously will have major international implications which must be addressed by policy-makers. No attempt has been made to address these implications in this report.

REFERENCES

1. Aquaculture Outlook and Situation, U.S. Department of Agriculture ERS, AS-3, April, 1982.
2. Atinmo, T., Pond, W. G., and Barnes, R. H., "Effect of Maternal Energy vs. Protein Restriction on Growth and Development of Progeny in Swine," J. Anim. Sci. 39:703-711, 1974.
3. Baile, C. A., "Regulation of Energy Balance and Control of Food Intake", in Digestive Physiology and Nutrition of Ruminants, Vol. 2, D. C. Church, ed. (O & B Books, Inc., Corvallis, OR, p. 291, 1979).
4. Baile, C. A., Della-Fera, M. A., and McLaughlin, C. L., "Performance and Carcass Quality of Swine Injected Daily With Bacterially Synthesized Human Growth Hormone," Growth 47:255-236, 1983.
5. Baker, P. K., Dalrymple, R.J., Ingle, D. L., et. al., "Use of Beta-Adrenergic Agonist to Alter Muscle and Fat Deposition in Lambs," J. Anim. Sci. 59:1256-1261, 1984.
6. Baldwin, R. L., and Allison, M. J., "Rumen Metabolism," J. Anim. Sci. 57:(Suppl. 2):461-477, 1983.
7. Bauman, D. E., and Elliot, J. M., "Control of Nutrient Partitioning in Lactating Ruminants," in Biochemistry of Lactation, T. B. Mepham, ed. (Elsevier Biomedical Press, Amsterdam, The Netherlands, p. 437, 1983).
8. Bellows, R. A., Short, R. E., and Staigmiller, R. B., "Research Areas in Beef Production," in Beltsville Symp.

Anim. Reprod. Agr. Res. 3, H. Hawk, ed. (Allanheld, Osmun and Co. Publ., Inc, NY. pp 3-18, 1979).

9. Bitman, J., "Status Report on the Alteration of Fatty Acid and Sterol Composition of Lipids in Meat, Milk and Eggs,"in Fat Content and Composition of Animal Products (National Research Council) National Academy of Sciences, Washington, DC, pp 200-237, 1976.

10. Cartwright, T. C., Gerrits, R. J., Kiddy, C. A., et al., "Animal Genetics and Reproduction", in Animal Agriculture: Research to Meet Human Needs in the 21st Century, W. G. Pond, R. A. Merkel, L. D. McGilliard, and V. J. Rhodes, eds., (Westview Press, Boulder, CO, pp 93-128, 1980).

11. CAST, "Antibiotics in Animal Feeds," Council for Agricultural Science and Technology, Report No. 88, Ames, Iowa, 1981.

12. CAST, "Diet, Nutrition, and Cancer: A Critique," Council for Agricultural Science and Technology, Special Publication No. 13, Oct., 1982.

13. CAST, "Food From Animals: Quantity, Quality and Safety," Council for Agricultural Science and Technology Report No. 82, Ames, Iowa, 1980.

14. Chung, C. S., Etherton, T. D., and Wiggins, J. P., "Stimulation of Swine Growth by Porcine Growth Hormone," J. Anim. Sci. 60:118-130, 1985.

15. Curtis, S. E., and Houpt, K. A., "Animal Ethology: Its Emergence in Animal Science," J. Anim. Sci. 57(Suppl. 2):234-247, 1983.

16. Dzuik, P. J., and Bellows, R. A., "Management of Reproduction of Beef Cattle, Sheep and Pigs," J. Anim. Sci. 57(Suppl. 2):355-379, 1983.

17. Echternkamp, S. E., Ferrell, C. L., and Rone, J. D, "Influence of Pre- and Post-partum Nutrition on LH Secretion in Suckled Post-partum Beef Heifers," Theriogenology 18:283-295, 1982.

18. Gargallo, J., and Zimmerman, D. R., "Effects of Dietary Cellulose Levels on Intact and Cecectomized Pigs," J. Anim. Sci. 53:395-408, 1981.

19. Harper, A. E., Dwyer, J., Brown, M. L., et al., "Human Nutrition," in Animal Agriculture: Research to Meet Human Needs in the 21st Century, W. G. Pond, R. A. Merkel, L. D. McGilliard, and V. J. Rhodes, eds., (Westview Press, Boulder, CO, pp 7-38, 1980).

20. Institute of Laboratory Animal Facilities and Resources, National Survey of Laboratory Animal Facilities and Resources, U.S. Department of Health and Human Services, Public Health Service, National Institute of Health, NH Publication No. 80-2091, National Academy of Sciences, Washington, DC, pp 36, 1980.

21. Kass, M. L., VanSoest, P. J., and Pond, W. G., "Utilization of Dietary Fiber From Alfalfa by Growing Swine. II. Volatile Fatty Acid Concentrations in and Disappearance From the Gastrointestinal Tract," J. Anim. Sci. 50:192-197, 1980.

22. Kolata, G, "Lowered Cholesterol Decreases Heart Disease," *Science* 223:381-382, 1984.

23. Koong, L. J., Nienaber, J. A., Pekas, J. C., et al., "Effects of Plane of Nutrition on Organ Size and Fasting Heat Production in Pigs," *J. Nutr.* 112:1638-1642, 1982.

24. Larson, W. E, "Crop residues: Energy Production or Erosion Control," *J. Soil and Water Conservation* 34:74-80, 1979.

25. Machlin, L. J., "Effect of Porcine Growth Hormone on Growth and Carcass Composition of the Pig. *J. Anim. Sci.* 35:794-800, 1972.

26. Montencourt, B. S., "Application of Recombinant DNA Technology to Cellulose Hydrolysis," *Biotechology* 1:166, 1983.

27. National Aquaculture Development Plan, *The Status of Aquaculture*, Joint Subcommittee on Aquaculture, Federal Coordinating Council on Swine, Engineering and Technology. Aquaculture Magazine 1984 Buyer's Guide, Washington, DC, pp 4-10, 1983.

28. National Center for Health Statistics, *Consumption Patterns in the United States 1971-1974*, Vital Health Statistics, Series 11, Public Health Service, Dept. Health, Education and Welfare, Hyattsville, MD, 1980.

29. National Center for Health Statistics, *Fat, Cholesterol and Sodium Intake in the Diet of Persons 1-74 Years*, United States Advanced Data No. 54, Vital Health Statistics, Public Health Service, Dept. Health, Education and Welfare,

Hyattsville, MD, 1979b.

30. National Center for Health Statistics, Overweight Adults in the United States, Advanced Data No. 51, Vital Health Statistics, Public Health Service, Dept. Health Education and Welfare, Hyattsville, MD, 1979a.
31. National Research Council, Aquaculture in the United States-Constraints and Opportunities, National Academy of Sciences, Washington, DC, 1978b.
32. National Research Council, Diet, Nutrition and Cancer, Committee on Diet, Nutrition and Cancer, Assembly of Life Sciences, NRC, National Academy of Sciences, Washington, DC, 1982.
33. National Research Council, Fat Content and Composition of Animal Products, National Academy of Sciences, Washington, DC, pp 243, 1976.
34. National Research Council, Nutrient Requirements of Coldwater Fishes, No. 16, National Academy of Sciences, Washington, DC, pp 63, 1981a.
35. National Research Council, Nutrient Requirements of Laboratory Animals, National Academy of Sciences, Washington, DC, pp 96, 1978a.
36. National Research Council, Nutrient Requirements of Warmwater Fishes, National Academy of Sciences, Washington, DC, pp 78, 1977.
37. National Research Council, Nutrient Requirements of Warmwater Fishes and Shell Fishes, National Academy of Sciences,

Washington, DC, pp 102, 1983.

38. National Research Council, Plant and Animal Products in the U.S. Food System, National Academy of Sciences, Washington, DC, pp 254, 1978.

39. National Research Council, Recommended Dietary Allowances, 9th Edition, Committee on Dietary Allowances, Food and Nutrition Board, National Academy of Sciences, Washington, DC, pp 185, 1980a.

40. National Research Council, The Health Effects of Nitrate, Nitrite and N-Nitroso Compounds, Committee on Nitrite and Alternative Curing Agents in Foods, National Acacemy of Sciences, Washington, DC, 1981b.

41. National Research Council, Toward Healthful Diets, Food and Nutrition Board, National Academy of Sciences, Washington, DC, pp 47, 1980b.

42. Oltjen, R. R., Ullrey, D. E., Ammerman, C. B., et al., "Animal Nutrition and Digestive Physiology," in Animal Agriculture: Research to Meet Human Needs in the 21st Century, W. G. Pond, R. A. Merkel, L. D. McGilliard, and V. J. Rhodes, eds. (Westview Press, Boulder, CO, pp 93-128, 1980).

43. Oltjen, R. R., and Dinius, D. A., "Production Practices that Alter the Composition of Foods of Animal Origin," J. Anim. Sci. 41:703-722, 1975.

44. Owens, F. N., and Bergen, W. G., "Nitrogen Metabolism of Ruminant Animals: Historical Perspective, Current

Understanding and Future Implications," J. Anim. Sci. 57(Suppl. 2):498-518, 1983.

45. Pearson, A. M., and Wolzak, A. M., "Salt - Its Use in Animal Products - A Human Health Dilemma," J. Anim. Sci. 54:1263-1278, 1982.

46. Pond, W. G, "Nutrition and Heart Disease: The Pig as an Experimental Model", in Pig Model for Biomedical Research, H. R. Roberts, and W. J. Dodds, eds. (Pig Research Institute of Taiwan, Chunan, Miaoli, Taiwan, Republic of China, p 43-59, 1982).

47. Pond, W. G., Mersmann, H. J., and Yen, J. T., "Severe Feed Restriction of Pregnant Swine and Rats: Effects on Postweaning Growth and Body Composition," J. Nutr. 115:179-189, 1985.

48. Production Credit Association, Agriculture 2000 - A Look at the Future, Battelle Press, Columbus, OH p 163-167, 1983.

49. Ravelli, G. P., Stein. Z. A., and Susser, M. W., New Eng. J. Med. 295:249-253, 1976.

50. Reid, R. L., and Klopfenstein, T. J., "Forages and Crop Residues: Quality Evaluation and Systems of Utilization. J. Anim. Sci. 57(Suppl. 2):534-562, 1983.

51. Ricks, C. A., Dalrymple, R. H., Baker, P. K., et. al., "Use of a Beta-Agonist to Alter Fat and Muscle Deposition in Steers," J. Anim. Sci. 59:1247-1255, 1984.

52. Ryther, J. H., "Mariculture, Ocean Ranching, and Other Culture-Based Fisheries, Bioscience 31:223-230, 1981.

53. Stanton, H., and Mersmann, H. J., "Swine in Cardiovascular Research," CRC Press, Boca Raton, FL (In press), 1985.

54. U.S. Congress, Office of Technology Assessment, Commercial Biotechnology: An International Analysis, OTA-BA-218, Washington, DC, January, 1984.

55. Varel, V. H., Pond, W. G., Pekas, J. C., et al., "Influence of High Fiber Diet on Bacterial Populations in Gastrointestinal Tracts of Obese- and Lean-Genotype Pigs. Applied and Environmental Microbiol 44:107-112, 1982.

56. Visek, W. J., "The Mode of Action of Growth Promotants by Antibiotics," J. Anim. Sci. 46:1447-1469, 1978.

57. Webster, A. J. F., "The Energetic Efficiency of Growth," Livestock Prod. Sci. 7:243-252, 1980.

Background Paper No. 6

Livestock Pest Control

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LIVESTOCK PEST CONTROL

Summary

Major insect pests cause losses to livestock and poultry of more than $2.5 billion. Some insects, primarily the blood feeders, are pests of all warm-blooded animals. Others are host-specific, although related species may prey on several classes of livestock. Losses may be direct (in terms of decreased livestock products) or indirect (insect-transmitted disease, secondary infections, predisposition to other diseases, irritation that causes unthriftiness, and cost of insect control).

New technology, particularly that which will affect livestock insects that are difficult to control, will be more expensive and will have a lower cost-benefit ratio than that of current technology. Progress in new technology in the science of veterinary entomology is relatively slow for the same reason that adaptation of existing technology is slow--there are few scientists (60) doing research. Several technologies show promise for controlling insect pests of livestock, however.

Although animal producers will continue to use insecticides for the immediate future, progress is being made in such areas as habitat management (pasture rotation and brush control for ticks), integrated pest management (biocontrol, sanitation, and waste management for fly control at feedlots and dairies), and use of pest-resistant breeds in cross-breeding programs (Indian crossed with European cattle).

For blood-feeding insects, research is directed at developing slow-release technology--whereby a chemical ingredient is formulated into a matrix that slowly erodes or vaporizes to release insecticide. Insecticide boluses remain in the stomach and slowly release insecticide which destroys manure-developing fly larvae.

The vaporized insecticides from eartags spread over the haircoat of an animal destroying insects that rest or feed on the animal. Eartags have been very effective and widely used for horn fly control and have improved face fly control on cattle. However, horn fly resistance to the pyrethroid insecticides used in eartags has become widespread.

The newest of these technologies, implants will have a limited effect for migratory, blood-feeding insects unless many producers join the control effort. Implants release insecticide directly into the bloodstream which destroys blood-feeding insects.

Recombinant DNA technologies will be used for the molecular cloning of desired antigens, toxins, enzymes, or other biologically important molecules for use as research tools or in the development of vaccines for blue tongue, anaplasmosis, and other diseases for which insects are vectors. In addition, this technology will enhance the study of molecular genetics and metabolic control in *Bacillus thuringiensis*, a bacterium pathogenic to some insects. Recombinant DNA technology will be used to clone insect antigens for screening potential vaccines or to test the effects of antigens on immunological response.

Advances in genetics will allow scientists to manipulate the reproductive capabilities of pest species. These advances include the sterile insect release method and chromosomal translocation, among others.

If technology already available were used on a wider scale, livestock losses from insects could be reduced by one-third ($700 million). This outcome would entail at least a doubling of current extension efforts in livestock entomology. The new methodology discussed above has the potential to reduce losses by another 15-25 percent, but at a lower cost-benefit ratio.

ANIMAL TECHNOLOGY WORKSHOP

Sponsored by
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Congress of the United States

Livestock Pest Control

by

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The information contained within this document is by necessity opinionated since it has been compiled by one author. The author has relied on data from six major publications (cited in the appendix) in addition to his own in preparing this document.

Introduction

Definition and scope: Several species of insects are considered major pests of livestock. Some, primarily the blood feeders, are pests of all warm-blooded animals. Others are host-specific although there may be related species that parasitize the several classes of livestock. The losses inflicted to livestock production by insects take several forms. Direct losses include reduction in animal weight gains, milk, egg and wool production, damage to hides, and even death. Indirect losses are much more difficult to accurately determine but include predisposition to diseases, diseases transmitted by insects, secondary infections, irritation and annoyance causing unthriftiness and cost of insect control. Drummond, et al (1981) estimates the total annual loss to cattle, sheep, swine and poultry production resulting from predation by the major insect pests to be in excess of $2.5 billion.

For the purpose of this report, the life cycle, economic impact and control methodology for each pest on each class of livestock will be discussed. Additionally, where applicable, livestock management systems and their relationship to pest control will be noted.

Cattle

Cattle grubs: There are two species, *Hypoderma lineatum* and *H. bovis*, the common and Northern cattle grubs, respectively. Life cycle: The grubs have one generation per year. The adult (heel fly) deposits eggs on the hairs of cattle in the spring or early summer. The larvae hatch and bore into the skin of the animal. The grubs spend 7 to 8 months as internal parasites migrating through the tissues of cattle. Eventually they arrive at the loin area of the back. At this point they encyst (warble), cut a breathing hole in the skin of the animal and complete their larval development. The mature grub emerges through the breathing hole, falls to the ground, finds shelter, pupates when enough heat units accumulate, and changes to the heel fly ready to start another life cycle.

Economic losses: Although cattle grub distribution is generally restricted to the range states of the Great Plains and West, annual losses still exceed $600 million. Heel flies cause cattle to stampede (gad) when they are depositing eggs. The extent of these losses are unknown. The effects of grubs as internal parasites are documented and so are losses in packing plants where grubby hides and the necessity of trimming grubby carcasses decrease the value of the slaughter animal. Packers dock cattle producers 5 cents/pound if grubs exceed 5/animal as a general business practice.

Grub control: The advent of systemic insecticides in the early 1950's (which are quite effective, can be administered by a variety of methods and are reasonably priced) makes it difficult to understand why grub losses remain so high. Several countries in Europe and several provinces in Canada have practically eradicated cattle grubs by following a rigid legislated annual control program.

Cattle management: The practice of weaning calves in the fall and/or moving cattle to winter range allows an ideal time for administering vaccination and treatment for cattle grubs because cattle are being worked for several purposes at one time. In a period of 3-years, we have been able to reduce the incidence of grub infestation by more than 95% by treating all calves remaining in the herd with a systemic insecticide at weaning time. Most feedlots treat all cattle coming into a feedlot for grubs as part of their routine herd health program.

Cattle lice: There are four blood feeding and one skin feeding species of cattle lice in the U.S. The blood feeders are *Haematopinus eurysternus* (short-nosed louse), *Linognathus vituli* (long-nosed louse), *Solenopotes capillatus* (the little blue louse) and *H. quadripertusus* (cattle tail louse); and the biting louse (*Bovicola bovis*) feeds on skin.

Life cycle: All species of cattle lice have gradual metamorphosis with feeding habits, appearance and behavior of juveniles similar to that of the adults. The life cycle requires about 24-days from egg to adult. The rate of reproduction increases during cold weather and declines in warm weather. Cattle lice are generally host specific, obligate parasites and spread by animal to animal contact. Eggs (called nits) are deposited on hairs of cattle in a species-specific manner.

Economic losses: Cattle lice may be the most underestimated economic insect pest of cattle. Estimated annual losses to the cattle industry exceed $125 million. Loss of weight, anemia, anemic abortion, predisposition to respiratory diseases and even death are attributable to cattle lice. Although the literature contains conflicting data on the degree of infestation necessary to be economic, there is general agreement that heavy populations cause severe losses. The greatest losses occur in areas with extreme winter climate. Because cattle lice are small and diagnosis may require restraining an animal, animal condition (rough, dirty, patchy haircoat) and animal behavior (severe itching) is often used as a guide for treatment. Unfortunately, much of the loss occurs before the animal reaches a condition suggesting a need for treatment.

Control: Every insecticide registered for use on animals is fairly effective on lice. However, few have toxic residues lasting long enough to kill all lice that hatch after treatment and most do not destroy eggs. Some animals seem to be "chronic or carrier" in that they seem to be very susceptible to heavy lice infestations. It is difficult to control lice on these animals, and they often serve as a reinfestation source after herd treatment. Culling of these animals will aid in control of lice in a herd.

In practice many cattle producers treat cattle in the fall for cattle grubs and assume this treatment also provides control of lice. While the systemics do reduce lice numbers dramatically, they may not prevent a build-up in populations in late December or January.

Animal management: As was indicated for cattle grubs, working range cattle in the fall provides an opportunity to treat cattle for lice. Feeder cattle are treated for both grubs and lice with one treatment of the same product when they enter the feedlot. There are data that strongly suggest that a high plane of nutrition such as received by feeder cattle will cause a natural decline in populations of lice.

Cattle mites: There are four species of mites that are of economic importance to cattle in the U.S. Psoroptes ovis, the scabies mite, and Sarcoptes scabiei and Chorioptes bovis, scab mites, are or may be subject to federal and/or state quarantine regulations. The cattle follicle mite, Demodex bovis, does not cause a scabby condition and is economically important only on dairy cattle in some locations.

Life cycle: The entire mite life cycle is spent on the animal and populations increase in the winter and decline in the summer. Life cycles average about 3-weeks from egg to adult. Mites are spread by animal-to-animal contact.

Economic losses: The scabies and scab mites are considered (legislated) a contagious disease and are subject to quarantine and treatment regulations of the Veterinary Services Division of the Animal and Plant Health Inspection Service (APHIS). Chorioptic mites are considered a "reportable" disease in some Northeastern dairy states.

Untreated cattle lose a great deal of weight, itch to the point of creating wounds by scratching and often die. Scabies outbreak in feedlots cost an average of $50/animal and losses average $25/animal for range cattle. The average annual losses to scabies in the U.S. is estimated to be in excess of $15 million.

The demodex follicle mite creates nodules that may be golfball size, but the overall economic impact is not great since this pest is usually restricted to the Northeast dairy states and only a few animals in a herd may have nodules.

Control: The scabies mites must be controlled according to federal regulations which require two supervised treatments 10 to 14 days apart for infested animals. Treatments in the past were dips or spray-dips with toxaphene, lime-sulphur or, in some cases, coumaphos. Recently, Ivomec (an injectable antihelminthic agent) has been approved for scabies treatment. In addition, any animals that might have come in contact with infested animals must be treated once. During outbreaks, state animal health officials may require treatment of all cattle coming into a state from outbreak areas.

Demodex control is probably impractical. Marketing animals with nodules may be in order. Repeated treatments with systemics may be the only method that will control demodex mites.

Stable flies: The stable fly, *Stomoxys calcitrans*, is a blood-feeding fly. The mouthparts of this fly are capable of penetrating the skin of cattle and other warm-blooded animals. It has complete metamorphosis. Eggs are deposited in wet, decaying, organic matter. Manure mixed with spilled feed or dirt and moisture (material readily found in and around feedlots and dairies) makes an ideal breeding media. Fly larvae feed in the breeding area, complete development, and pupate; within about a week, the new adults emerge. The life cycle is complete in about

3 weeks during the summer. The stable fly overwinters in breeding areas, below the frostline, as a slowly developing larva until temperatures rise in the spring. The larvae then pupate; and when the adults eclose, they give rise to the first generation of the spring. Females live 2 to 4 weeks and deposit 200 to 600 eggs in their life time.

Economic losses: The stable fly is a major pest of feedlot cattle in the Midwest and dairy cattle in the Northeast. It is also a general pest of cattle in the Southeast. Milk losses of 40% and weight reductions of 0.48 lb/day have been noted. Annual U.S. losses are estimated to be in excess of $398 million.

The bite of the fly, because its mouthparts pierce the skin, is quite painful. Cattle being fed upon by stable flies bunch--each animal trying to protect its front legs, the favored feeding site of stable flies. The peak population of stable flies (late July and august) may coincide with a weather index danger period for cattle (i.e. high temperature and humidity) which may increase losses.

Control: Feedlot sanitation is the key to stable fly control. Initially, considerable labor and equipment costs may be incurred. Poor drainage, improper mounding, faulty waterers and poorly designed or maintained feeding systems all contribute to fly production. A feedlot design which considers these factors will contribute to fly control and to the control of rodent and bird pests.

An integrated pest management (IPM) system has been developed which utilizes proper sanitation and intelligent use of insecticides which can be adapted to any class of confined livestock management. Biological control has shown some promise and could complement chemical control. However, parasitic wasp species being sold commercially have failed to control flies in Midwest feedlots.

Stable flies are also a pest of pastured cattle in the eastern half of the U.S. The losses to these cattle are unknown but probably dramatic, and there is no adequate control for stable flies on pasture cattle.

Horn flies: The horn fly, Haematobia irritans, is a small blood-feeding fly. As an adult, it is an obligate parasite in that it remains on the animal almost constantly. Horn flies have complete metamorphosis. Eggs are deposited in fresh cow manure where larvae develop, move to soil and pupate. The entire life cycle can be completed in 10 to 14 days during the summer. Horn flies overwinter as pupae. The short duration of the life cycle and the high number of eggs deposited/female may cause populations to reach levels of 1000 flies/animal.

Economic losses: Because of its wide distribution and high numbers, losses from the horn fly are estimated to be in excess of $730 million annually in the U.S. This figure places the horn fly as the most important livestock insect in the U.S.

Control: Slow-release, insecticide-impregnated eartags, developed in 1981, were rapidly adopted by producers of range and pasture cattle. Over 50 million tags were sold in 1983. The tags have provided horn fly control in excess of 99%. Unfortunately, resistance has developed to fenvalerate and permethrin (the synthetic pyrethroid insecticides used in the tags).

Forced-use of dust bags and oilers (self-treatment devices) can provide excellent horn fly control. Feed additives such as Rabon and methoprene may also provide good fly control, particularly if cattle are isolated and the vegetation is not salty.

Animal management: The management of cattle on range land is not complementary, in many instances, to horn fly control. If cattle water from ponds or streams, it is difficult to use forced-use, self-treatment devices. Some producers have already eartagged animals with identification tags and do not want to use another tag. This is particularly true of purebred cattle producers.

Face flies: The face fly, Musca autumnalis, is not a blood feeding parasite unless blood is provided by an existing wound. It feeds on animal mucous and dung liquids. It is a very persistent feeder around the eyes and nostrils of cattle and horses.

Life cycle: The life cycle of the face fly is similar to that of the horn fly. However, the life cycle takes about 24-days to complete in the summer and it overwinters in sheltered places as an adult. The length of the life cycle limits the distribution of the face fly. In the hot open range areas, manure dries too rapidly to allow the face fly to complete its life cycle.

Economic losses: Losses from the face fly to the cattle industry is estimated at $53 million annually. Recent studies (under artificial, confined conditions in the case of beef cattle and under more natural conditions with dairy cattle) did not show direct weight gain or milk production losses between fly-infested and fly-free cattle. Several studies have shown the face fly to be a vector of pinkeye, Moraxella bovis and the feeding habits of the fly around the eye causes wounds which provide an avenue for eye pathogen establishment. The eyeworm, Thelasia sp., is spread to cattle and horses by the face fly. In addition, there is evidence that Infectious Bovine Rhinotracheitis (IBR) virus can be transmitted by face flies.

Control: The same methods and products used for horn fly control are also recommended for face fly control. However, face fly control is difficult to obtain because the face fly feeds around the head, a part of the animal difficult to treat. A second, compounding problem with treatment of the face fly is that they remain on the animal only a short period of time and, in fact, can survive without ever feeding on an animal. However, females use animal tears and mucous as a source of protein for egg development.

Control of face flies, below an economic threshold, probably requires more than one method; for example, eartags and forced-use of oilers or dust bags or sprays plus eartags or oilers.

House fly: The house fly, Musca domestica, is present at any farm that produces livestock. Its life cycle is very similar to that of the stable fly except that it does develop in fresh manure and does not take blood meals. Two weeks is adequate for the life cycle in the summer.

Economic losses: Losses inflicted by the house fly are difficult to assess. Animal production studies indicate no loss, for either cattle or swine, by fly infestation. The house fly is capable, however, of mechanical transmission of many diseases to both animal and man. The fly is also implicated in many agricultural nuisance lawsuits.

Control: House fly control methods are about the same as for stable flies. Sanitation is the key for control of both species. If breeding source reduction isn't incorporated into a control program, fly production will simply override the effects of insecticides.

Mosquitoes, black flies, horse and deer flies and Culicoides: This complex of the order Diptera (flies) are aquatic or semi-aquatic for at least part of their life cycle. Eggs are deposited in either wet soil or water, and larval development is usually completed in that environment. The adults, or at least the female adults, are blood feeders. Many species of the various groups are vectors of animal diseases, because of their blood feeding habits.

The life cycles vary dependent on species. Many mosquito species overwinter as eggs. There are usually several generations per year. They have complete metamorphosis. The same is true of *Culicoides*. Black flies, deer flies and horse flies may have only one generation per year dependent on the species.

Economic losses: Mosquito losses to the cattle industry are estimated to be $40 million annually. Losses from the other groups are unknown. However, losses must be substantial because the horse and deer flies are vectors of anaplasmosis, and animals bitten by them are obviously in pain. *Culicoides* transmit blue tongue virus, a disease that may cause abortions. The virus is shed in bull semen--blue tongue positive bulls are rejected as semen donors. Black flies are very annoying and, as is true with all of the aquatic diptera, are a very important problem in Southeastern states because of the numbers that develop. Young calves may become asphyxiated from inhaling black flies and mosquitoes. The black fly secretes a toxic substance that may act as an anticoagulant and causes an antigenic reaction in many animals.

Control: The aquatic complex of insects are very difficult to control; there are no practiced recommendations for horse fly or deer fly control. Drainage of breeding areas, in the case of mosquitoes and to a lesser degree *Culicoides*, helps in control. Release of chemicals, upstream from black fly breeding areas and in impounded mosquito breeding areas, provides some control but is not used on a wide scale. All of the control methods used for control of flies may have some effect on the aquatic, blood-feeding insects but couldn't be considered control. In general, there is no practical method to protect livestock from these insects, because their breeding areas are difficult to identify or so abundant that control would be very costly.

Animal management: The economic loss from the aquatic insect complex is so severe that many producers do not pasture cattle along fly breeding waterways in the summer.

Ticks: There are about 20 species of ticks that are of veterinary importance in the U.S. Of these, six cause major economic losses. Ticks in the U.S. exhibit two basic life cycles. Some are one host species that attach to a host as larvae, begin feeding and complete the nymph and adult stages on the same host. Mated, fully engorged females drop from the host and initiate another cycle. The three-host ticks feed on two separate hosts before adults finally attach to cattle, or other large animals, as a third host. The winter tick is an example of the one-host tick; and the lone star tick and the Gulf Coast tick are examples of the three-host tick. The major tick species are *Amblyomma americanum* (lone star tick), *A. maculatum* (Gulf Coast tick), *Dermacentor albipictus* (winter tick), *D. occidentalis* (Pacific Coast tick), *Ixodes scapularis* (blacklegged tick), *Otobius megnini* (spinose ear tick) and *Ornithodoros coriaceus* (pajaroella tick).

Boophilis annualatus, a one-host tick, is potentially important in the U.S. because it is the vector of Texas cattle fever (Bovine piroplasmosis), a protozoan blood parasite. Although the disease is technically eradicated from the U.S., this tick species occasionally is found in Texas counties bordering Mexico. The U.S. and Mexico have had a cooperative program designed to eradicate the disease in Mexico as well as the U.S.

Economic losses: Losses to the cattle industry in the U.S. from ticks are estimated to be in excess of $275 million annually. Much of the cost is because treatment is ineffective and, thus, needs to be repeated often. They are also implicated as disease vectors.

Control: The primary method of treatment for ticks is dipping or spraying. For species that have more than one generation or individual development that occurs over a long period of time, it is necessary to treat at about 2-week intervals. Eartags have been efficient in control of the Gulf Coast tick. Resting pastures, pasture rotation and brush control (although often expensive) is being used in some areas of the country. Indian breeds of cattle are resistant and are used to some extent in tick infested areas. Animal management: Where possible, keeping cattle away from areas infested with tick nymphs causes high tick mortality, because they need a blood meal to survive.

Swine

Most of the blood feeding Diptera discussed in the cattle section will also feed on swine, but the incidence of attack is much greater for cattle. There have been very few studies made to determine economic losses to swine from insects. Our studies show no effects of house fly infestation in the absence of disease; but the ability of the house fly to transmit many of the major swine diseases, such as cholera, is well documented. Because urban sprawl has infringed on agricultural areas, many lawsuits against swine producers cite unpleasant odor and house fly breeding.

Lice and mange mites are considered the major insect pests of swine. There are numerous studies on these pests, primarily because they remain on the animal as obligate parasites and are easier to study than species that leave the animal after feeding (migratory). Immatures and matures have the same feeding habits.

Hog mange mites: Although there are two species of mites, Sarcoptes scabiei suis, the mange mite, and Demodex phylloides, the follicle mite, only the first species is of economic importance. Sarcoptic mange mites burrow through the skin of hogs, leaving tunnels in which females deposit eggs. Infestations increase during winter; and once wounds and scabs develop, the reproduction cycle continues to increase. Egg to adult takes an average of about 2-weeks.

Economic losses: Mange mites cost swine producers over $75 million annually. Mites cause severe itching and swine create wounds from scratching. Lesions develop at the wound site, usually around the head, eyes, nose and inside the ear. The ear is often the starting point for mange and, thus, not detected early. About 25% of hogs examined on kill floors in Nebraska and Indiana had mites inside the ear.

Control: Control is difficult to achieve. The only efficient method has been the repeated application of lindane, a long residual insecticide; though Ivermectin, a new injectable product, seems to be quite effective.

Animal management: A disease control program, called SPF (Swine Pathogen Free), has been in use for several years in major swine producing states. Basically, it assures producers that breeding stock are free or immune to several serious swine diseases. As a part of that program, the animals must be free of lice and mites. Many producers not involved in the production of breeding stock have adopted this system.

Hog lice: The hog louse, Haematopinus suis (L.), is host specific. The life cycle is similar to all louse species, with completion of the cycle in about 24-days. The hog louse is the largest of the louse species and thus easy to see on swine. They decline in numbers in the summer and increase in the winter.

Economic losses: Estimated losses are in excess of $150 million.

In Indiana, a major swine production state, the incidence of hog lice averages 17%.

Control: Hog lice are not difficult to control with applications of insecticides to the animal. Two treatments may be required because insecticides do not kill louse eggs. Ivermectin is very effective for hog lice.

House flies: The life cycle, economic loss and control has been discussed in the cattle section. House flies are especially attracted to swine production units for breeding. Subsequently, great numbers of house flies develop; and, because flies transmit swine diseases and travel from farm to farm, there is a continual threat of a disease outbreak which could become an economic disaster to a swine producer.

Sheep and Goats

The sheep and goat industry has declined because of competition between wool and synthetic fibers. Goat production is limited to the southwest except for small individual flocks. Nevertheless, losses are estimated to be in excess of $54 million from sheep keds and nose bots. Biting flies, discussed in the section on cattle, would also be pests of sheep. *Culicoides* are particularly important because sheep are very susceptible to blue tongue (BTV). As with all domestic animals, sheep have several host specific species of lice. Scabies mites have been reported as eradicated from sheep in the U.S. Wool maggots, immatures of blow flies (primarily *Cochliomyia macellaria*, *Phaenicia sericata* and *Phormia regina*), create or expand wounds on sheep. Only bots, keds and blow flies will be discussed in this section, since pests of sheep which also affect cattle have been discussed in that section.

Sheep ked: The sheep ked, Melophagus ovinus, (often called sheep tick) is a wingless fly. It is an obligate parasite which spends its entire life cycle on sheep. This insect has an unusual reproductive system--an egg hatches within the uterus of the female, the larva feeds on nutritive glands of the female, is birthed by the female when larval development is completed and then forms a puparium. Only one larva develops at a time. The entire life cycle takes about 30 days. Peak numbers occur in the winter.

Economic losses: The sheep ked, a blood-feeder, affects weight gain performance by 8 or 9 lbs. between birth and weaning. Wool quality and quantity is affected by sheep ked. Feeding sites cause a condition in sheep skins called cockle which decreases the tensile strength of the skin and causes hide buyers to downgrade the value of the hide.

Control: Several insecticides and methods of application are effective in control of sheep keds. In practice, sheep are usually treated at spring shearing; however, much of the economic loss has already occurred before shearing.

Sheep nose bot: The nose bot, Oestrus ovis, a fly which deposits living larvae near the nostril of host animals. The larvae migrate to sinus cavities of the nasal passages where they feed and develop. When larval development is complete the larvae migrate down the nasal passage, drops to the ground and pupates. When pupation is complete, the adult fly emerges. In the south, there may be a second generation; in the north, there is only one generation per year.

Economic losses: Although there are few economic studies, damage from the nose bot is two-fold. Larvae in nasal passages and sinuses cause irritation, and sheep rub their noses against feedbunks, fences or the ground. The fly is also annoying when it attempts to deposit larvae. Sheep under attack run back and forth, stamp their feet and hold their nose close to the ground. Normal grazing and resting doesn't occur when sheep are pestered by the bot fly.

Control: There currently are no registered insecticides for control of the sheep nose bot.

Blow flies: Blow flies, regardless of species, have the same life cycle. The flies are attracted to bacterial activity in the wool--wounds, wool contaminated by urine, sweat and feces all allow bacterial development. The life cycle of all species is approximately 2-weeks. There are several generations per year; and, dependent on the species, they overwinter as adults, larvae or pupae.

Economic losses: Wool maggots, also called fleece worms, cause lesions and irritate sheep--they go off feed, lose weight, become unthrifty and in poor condition. If left untreated, death may occur in a few days.

Control: Sheep can be sprayed with a residual animal spray at the time blow flies are present which provides some protection. If sheep are sheared after flies are present, spraying or dipping may be necessary to prevent flies from feeding on shearing wounds.

Animal management: Clipping soiled fleece from the crotch area and careful handling of sheep to avoid cuts are good practices. Shearing early helps insure that shearing wounds heal before blow fly season.

Poultry

As indicated for other livestock classes, many of the flies affecting cattle will also affect poultry. Losses are estimated to be in excess of $100 million annually. The major insect pests of poultry are ectoparasites and filth flies. Major ectoparasites include fowl mite, red mite, lice, chiggers and the black fly. The filth flies include house fly, several kinds of blow flies and *Fannia sp.*, the little house fly. Much of the loss to poultry from insects can be attributed to the cost of control (sanitation and insecticides).

Northern fowl mite: The Northern fowl mite, *Ornithonyssyus sylviarum*, has a somewhat complicated life cycle. Eggs are attached to feathers. The first post-egg stage, a six-legged larvae, doesn't feed and is followed by two nymphal stages--only the first feeds. The second stage nymph molts and changes to the adult stage. This life cycle may be completed in 1-week and all life stages can be found around the tail, legs, wings and neck of infested birds.

Economic losses: The Northern fowl mite is a blood-feeding parasite. Higher populations are present in the winter. Heavy populations cause weight loss, decreased egg production and a sharp decline in fertility of males, of particular importance to artificial insemination programs.

Control: Insecticide applications to the ground or directly to birds is about the only control method in practice. Once infestations are present, it usually takes repeated applications to end the problem.

Chicken mite: The chicken mite, *Dermanyssus gallinae* (also called red mite), is hard to distinguish from the Northern fowl mite. Life cycles are similar, except the chicken mite spends considerable time off the host and both nymphal stages feed on blood. This mite is restricted to tropical or temperate zones. Losses and control would be similar to that of the fowl mite.

Chicken lice: The body louse, *Menacanthus stramineus*, and the shaft louse, *Menopon gallinae*, are the most common of the six or seven species of lice that infest poultry in the U.S. All species are biting lice. None are blood-feeding.

Economic losses: Irritation by the pest, from mouthparts and sharp claws, can cause death of young chickens and turkeys; and adults are weakened, go off feed and have reduced weight or egg production.

Control: Spraying or dusting each bird is the only feasible way of obtaining control.

Filth flies: Although over 50 different fly species breed in chicken manure, only a few species reach numbers high enough to be classified as pests. The life cycle of most species is similar to that of the house and stable fly discussed earlier.

Economic losses: Most of the economic effect is due to disease transmission and nuisance.

Control: Proper waste management, screened facilities and the use of residual, knockdown and feed-through insecticides are the main methods of filth fly control. In some well managed confined systems, biological control, using beetle predators and wasp parasites, has been successful.

Major Lines of Research

Blood-feeding Insects

The slow-release concept is the technological formulation of a chemical ingredient into a matrix which slowly erodes or vaporizes to release the chemical. Insecticides, such as Ivermectin or the systemics, could be slowly released into the blood stream. Growth hormones used in cattle production are examples—the product is implanted in the ear and, over a 100- to 200-day period, is released at a constant level.

Cattle Grubs

Because of behavioral characteristics, the cattle grub is a candidate for sterile male releases. Male heel flies emerge earlier than females and locate in aggregation sites where they wait for the females. The biggest deterrent is that cattle grubs can't be reared artificially. Cooperative research between the U.S. and Canada on the integration of systemic insecticide practices with sterile male releases is in the second year.

The screwworm eradication program is a prime example of the successful employment of new methodology with the cooperation of federal and state research and regulatory and commodity agencies and organizations. Although several factors existed with the screwworm situation which are not present with most other insect parasites, it nevertheless stands out as one of the most successful programs in the 20th Century.

The screwworm was unique in that its distribution in the U.S. was limited by climate, it mated only once, cobalt irradiation caused sterility but didn't affect the mating competitiveness; and perhaps most importantly, the screwworm killed cattle and occasionally people. This last fact enabled the cattlemen's organization and the scientists to influence Congress to provide money enough to attempt the eradication program. Needless to say, the concept and research leading to the success of the concept must be considered a hallmark in the conversion of basic research to applied use in the field of biology.

Range Cattle Insects

Eartags impregnated with a slow-release insecticide have been very effective for horn fly control and have improved face fly control. This technology is only in its second year. Cattle producers rapidly accepted the concept; and in 1983, about half the cattle in the U.S. were treated. Two major eartag companies sold more than 30 million tags.

A problem has developed in eight states with resistance of horn flies to the synthetic pyrethroids used in the tags. In addition, one state indicated the development of resistance in face flies. Entomologists from various parts of the U.S. have outlined a management program which, hopefully, will prevent or slow the development of resistance.

There are conflicting opinions on this program. Some scientists feel that, since Canadian and Nebraska research suggests that the economic threshold for horn flies is more on the order of 200 to 250 flies/animal rather than 50 (a figure long used in literature), it isn't necessary to obtain 99% control. Further, they feel that resistance will develop more rapidly when 95% or better of the population is controlled than when only 75 to 80% control is achieved because a much higher percentage of susceptible individuals remain in the population. These concerns may be settled in the next couple of years with research.

In the range areas of Nebraska, use of insecticide eartags is so widespread that horn fly populations, even on untreated cattle, have been reduced at a magnitude of over 50%. Horn flies, although obligate parasites, do migrate between animals and herds. This widespread acceptance of the eartag method means that a great deal of commercial, state and federal research will be devoted to solving the resistance problem using better chemicals and better slow-release mechanisms.

Integrated Pest Management

The use of a systems approach to animal health is far behind the plant science program; however, integrated reproduction management, integrated pest management and integrated herd health management are being combined to form a systems management plan for livestock production. The North Central states are utilizing this concept to develop an animal herd health management model for the purpose of prioritizing research needs for confined or feedlot cattle (NCS-3). The Southern Region (S-181) is planning similar research for range cattle insects.

Current State of the Art

For the immediate future, animal producers will continue use of insecticides against livestock insect pests. However, progress is being made in areas such as habitat management (pasture rotation and brush control for ticks), integrated pest management (biocontrol, sanitation and waste management for fly control at feedlots and dairies), use of pest resistant breeds in cross-breeding programs (Indian crossed with European cattle) and better technology in terms of insecticide application methodology (slow-release). There is also considerable research being conducted on development of vaccines for blue tongue, anaplasmosis and other diseases for which insects are vectors.

Legislated control, although distasteful in concept, has proven effective in terms of scabies in the U.S. and cattle grubs in Europe and Canada. Perhaps more acceptable and practical is the use of commodity organizations, with assistance from extension, for organizing area control programs for specific pests.

Feed additives, boluses and implants under certain conditions show promise, particularly if the use is based on a wide-area control concept. These insecticide delivery systems will have a limited effect for migratory insects unless a large percentage of producers join in the control effort.

Monoclonal antibodies allow for enhanced recognition, quantitation, and purification of antigenic molecules. Monoclonal antibodies can be used to screen for bacterial clones producing target proteins that were engineered by recombinant DNA methodology.

Recombinant DNA methodologies will be used for the molecular cloning of desired antigens, toxins, enzymes, or other biologically important molecules for use in vaccine development (for ectoparasite control) or as research tools for further studies. In addition, recombinant DNA methodology will enhance the study of molecular genetics and metabolic control in Bacillus thuringiensis, an important entomopathogenic bacterium. Recombinant DNA technology will be utilized to provide a convenient source of test antigens cloned in bacteria from target insects to be used in screening potential ectoparasite vaccines, or to test the effect of the antigens on host immunological response.

The combination of immunological findings with regard to understanding the host parasite interactions between ectoparasitic insects and their animal (or human) hosts, and the ability to produce massive quantities of antigenic or regulatory proteins which have been identified as immunologically important, will result in the ability to utilize recombinant DNA technology to produce these proteins, or enzymes to make nonproteinaceous products. This could result in the availability and commercialization of animal vaccines for control of ectoparasites.

Advances in genetics will allow for the manipulation of pest species to interfere with their reproduction capabilities. Several techniques of genetic control are available and each of these should be evaluated for potential use with external parasites. These areas of genetic control include the sterile insect release method (SIRM), cytoplasmic incompatibility, chromosomal translocations, conditional lethals, compound chromosomes, meiotic drive, and incompatibility due to chromosomal factors.

Major Breakthroughs Needed

There are several obstacles to the progress of research which could provide technology necessary for major breakthroughs in livestock insect control.

1. The cost of livestock insects is in the neighborhood of $3 billion annually. Insects are very successful parasites in that they seldom kill their host and damage may be unnoticed or ignored. Losses inflicted by insects are insidious. They may be on the order of 10- to 20-lbs/animal and less for sheep, swine and poultry. As a result, losses often occur before the pest or its damage is apparent or, because losses aren't too severe, control measures are not taken.

2. In the case of diseases that cause heavy losses (IBR virus, pinkeye and anaplasmosis) current methods of control of the vector insects (Culicoides, face fly, Tabanids) are inadequate. A breakthrough for this situation is apt to be in the development of vaccines for the disease rather than a method for control of the insect.

3. Because insect losses (although real and very expensive to animal agriculture) are not dramatic, research and extension support is very inadequate. The USDA and State Experiment Stations have less than 60 scientists conducting research connected with livestock insects, and their combined research budget totals less than $5 million/year. In contrast, over 500 scientists, with a budget totalling more than $50 million, conduct research associated with plant pests. Many Land Grant Institutions have only part-time research appointments with time divided between teaching and research. Perhaps even worse than lack of research staff is the lack of extension effort. Extension entomologists have been allotted little time for livestock insects. As a consequence, insects (such as the cattle grub) still inflict heavy losses.

With intensive extension efforts, losses suffered from insects by the livestock industry could be reduced by one-third. The technology and methodology is available to accomplish that goal, within a 3-year period, by doubling the extension effort and increasing the research effort by one-third. Savings would be in the magnitude of $1 billion/year. The total increase in funding would be small compared to the potential savings.

Institutional Arrangements

Currently, research on livestock insects is being conducted by both Land Grant Institutions and federal laboratories. Very little research is conducted outside the confines of these two systems. Research is often cooperative between state and federal scientists within the state or neighboring states where federal laboratories are located (Nebraska, Florida, Texas). In addition, regional research funds have provided an opportunity for cooperative research between states (North Central Regional Project NC-154 and the Southern Regional Project S-181) that has greatly accelerated research progress. The investment of more money into regional research would provide rapid and dramatic returns because of the few scientists working on the problems.

Commercial companies provide some support funds for Land Grant Institution scientists (primarily for field trials on the efficacy of new insecticides). However, this support is relatively small because the sale of livestock insecticides is less than 4% of the total market.

Competitive animal health grants provide little funding for livestock insect research. In the past few years, less than 4% of these funds have been allocated to external (insect) and internal (worm) parasites.

Likely Timing of Commercial Introduction

Good livestock producers adapt new procedures or concepts very rapidly, if they meet certain criteria. The system must coincide with their livestock management scheme and must be reasonably-priced, effective and easy to use. A striking example is the insecticide impregnated eartag. Over 50 million were used in the second year of availability. In this case, extension efforts in a few states caught the attention of the media on a national basis. Producers were well informed of these products even before they were on the market.

Potential Primary Impacts

As discussed in the breakthroughs needed section, there is a potential for the reduction of losses from insects to livestock commodities by one-third ($1 billion) with widescale use of technology already available. This would require at least a doubling of the current extension effort in livestock entomology. IPM demonstrations at feedlots, dairies, swine and poultry units would be the best method of education. Pest control demonstrations, when weight gains between treated and untreated animals are compared, would make a tremendous impact on local producers.

The reduction of the cost to the livestock industry by one-third would have a tremendous effect on the agricultural economy. Economically, the investment-benefit ratios for livestock insects range from 1:2 to 1:8 with an average of 1:4. Thus, the investment of $150 million for livestock insect control should provide a return of $1 billion.

New methodology, discussed earlier in this text, has potential of reducing losses by another 15-25%; but in all probability, the cost of new technology would mean a cost-benefit ratio on the order of 1:2 rather than 1:4, as is the case with existing technology. New technology, which would provide adequate area control of ticks and the aquatic complex of insects, seems to be nonexistent. Even if vaccines are developed to prevent the diseases for which they are vectors, their economic impact would still be severe. There is, of course, considerable biological research being conducted on insects in terms of lethal genes, genetically incompatible subpopulations, immunological systems in animals to insects, biological control, and cellular biology dealing with insect biochemistry (producing the juvenile hormones, the chitin inhibiting enzymes and sex pheromones). These new systems haven't been very promising for area control of livestock insects, with the exception of the use of sterile insects for the successful elimination of the screwworm (*Cochliomyia hominivorax*) from the U.S. Those that have been effective for livestock insect control (methoprene, a growth regulator, and the CIBA-Geigy chitin inhibitor) are really little different than an insecticide in terms of cost. In fact, they simply compete with products already on the market that also control flies.

Supportive References

Drummond, R. O., G. Lambert, H. E. Smalley, Jr., and C. E. Terrill. 1981. Estimated Losses of Livestock to Pests. [In] CRC Handbook of Pest Management in Agriculture. Vol. I. CRC Press, Inc., Boca Raton, Florida. pp. 111-128.

Proceedings: Workshop on livestock pest management. J. B. Campbell, Coordinator. R. J. Sauer, Administrative Advisor. Kansas State University. March, 1979.

Symposium: Systems approach to animal health and production. 1981. F. W. Knapp, Editor. University of Kentucky, March, 1981.

Proceedings: North Central States Livestock Insect Pest Management Workshop, University of Nebraska. October, 1983.

Harwood, H. F. and M. T. James. 1979. Entomology in Human and Animal Health. 7th Edition. MacMillian Publishing Co., Inc., New York, NY. 548 pp.

Greenberg, B. 1971. Flies and Disease, Vol. I and II. Princeton University Press, Princeton, New Jersey. 1303 pp.

Background Paper No. 7

Environment and Animal Behavior

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ENVIRONMENT AND ANIMAL BEHAVIOR

Summary

The effects of environment on animal well-being have become ever more important with the trend toward production systems that confine a large number of animals together in a more artificial environment. Confinement simplifies the environment, reducing an animal's opportunities to alter its surroundings to advantage. While such intensive systems increase production per unit of labor input or space, they can be detrimental to animal function and performance.

The advent of intensive production systems changed the relative importance of various environmental factors as well as the strategies to improve animal production through the application of technology. New technologies likely to emerge by 2000 as a result of current research lie in the areas of energy conservation, optimization of total stress, stress-altered disease resistance, and photoregulation of physiological phenomena.

Feed and fuel--sources of energy--account for much of the cost of animal production. Although the tradeoffs between feed and fuel have been quantified for most species, the integration of additional research will result in further energy savings. For example, environmental temperature management schemes developed in an era of cheaper fuel are too luxurious today. There has been a tendency to maintain constant environmental temperatures even though the animals evolved naturally in a cyclical thermal environment. In one experiment young pigs were allowed to regulate their own environmental temperatures. They inserted a daily 20° F. fluctuation of warm afternoons and cool nights, resulting in unchanged pig performance but a 50-percent reduction in fuel use during cold weather. Lowering thermostat settings to parallel age-dependent changes in thermal requirements have also been found to save fuel. In some cases cooler surroundings spur appetites, so performance actually increases. Cost-effective, low-maintenance designs of heat exchangers and solar heating systems will effect further energy savings.

Either too much or too little environmental stimulation can have deleterious effects on the performance, health, and well-being of agricultural animals. To optimize total stress more must be learned about how stress acts on and is perceived by animals. Devices that animals can use to regulate certain environmental factors are already being recommended to farmers. Computerized sensing devices and control equipment will make biofeedback-linked automation of environmental regulation a reality in animal agriculture.

Researchers are also investigating how the environment influences specific mechanisms of immunity to disease. A variety of common environmental stressors--temperature, crowding, mixing, weaning, limit-feeding, noise, and movement restraint--are known to alter animals' defenses against infectious agents. New techniques in basic science, coupled with more traditional neurobiological, endocrinological, and immunological approaches, can yield a better understanding of how stressors influence regulatory signals among lymphoid cell subpopulations.

The regulation of light is of particular interest in animal production. The advent of photoperiod management revolutionized the poultry industry 40 years ago. Light is managed in poultry confinement operations so that it stimulates poultry growth. In the last 2 decades the effects of photoperiod management have also been characterized for sheep reproduction. Although the results of similar studies on cattle and swine have been less definitive, some results have been encouraging: Under controlled lighting, sows weaned heavier piglets, cows yielded more milk, and lambs grew faster. Experiments now in progress will produce information immediately applicable to animal production.

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CHAPTER 1

INTRODUCTION

Applied Ecology in Animal Agriculture

Environmental and hereditary forces shape an animal's life, thus they also determine the nature of animal production. The physical, chemical, and biological elements of the surroundings may either promote or impair animal performance by directly or indirectly facilitating or inhibiting productive and reproductive processes.

Environmental aspects of animal management are more important now than ever before with the trend lately to intensifying animal production in space and in time. Labor requirements are less in intensive animal production systems. Reduced need for land and easier waste management are other advantages. Also, environmental modifications that form an integral part of most confinement systems make year-round operations possible in many enterprises, thus seasonal cycles are not as marked as before. Intensive production systems may be detrimental to animal function and performance even though they increase production per unit of labor input or space. Confinement simplifies the environment, reducing the animal's opportunities to choose, alter, and use its surroundings to advantage. These restrictions are not necessarily beneficial. Confinement also complicates the environment in ways that may intensify counterproductive impingements. Yet the trend to more intensive production systems will continue so long as the plus factors outweigh the drawbacks.

The advent of intensive animal production systems changed the relative importances of respective environmental factors as well as the strategies to improve animal production through application of new technologies. In the more natural environments of extensive animal production, thermal factors and light are generally most important, and little attempt is made to alter them. Instead, production schedules are designed with seasonal differences in mind. In intensive systems, thermal and light factors are controlled, as are certain social and behavioral elements as well as factors that influence health. In addition to fitting management schemes and animals to the environment, there can be attempts to alter the surroundings to meet the animal's needs, to stimulate performance as well as provide protection against climatic rigors.

Scope of Applied Ecology in Animal Agriculture

Animal Environment. Animal environment is complex. Its individual elements not only vary in time and space, but they interact. The existing climate and weather--the macroenvironmental elements--set limits on all animal production systems. Mesoenvironment consists of local features--nearby topographical forms, vegetation, and farmstead structures. Both macro- and mesoenvironment help determine microenvironment, the hundreds of environmental elements which the animal actually experiences: thermal factors, light, other electromagnetic forces, air pollutants, sound, social factors (including humans), other behavior-inducing elements, and infectious agents. Also, factors such as water availability, altitude, and stray voltage are important in some settings.

Animal Adaptations. Animals must cope successfully with environmental impingements or else succumb to them. An animal's heredity determines its capacity to adapt, and thus and the environments the animal has experienced previously determine its adaptation status at any given time. Environmental stress permits genetic mutations having adaptive utility to be realized and fixed in the population. Also, several kinds of adaptation are induced by environmental stressors: acclimation--compensatory reactions to a single stressor, usually in an artificial situation; acclimatization--responses to stressful environments, such as in nature, in which several factors vary at the same time; and habituation--responses to certain repetitive stimuli diminish over time. Animals adapt by means of changes in functions, structures, immune traits, and behaviors. In the overall scheme, adaptations ensure an animal's survival. Requirements for short-term health and for productive and reproductive processes may be compromised so sheer survival needs can be served: reactions to stress alter internal functions, divert nutrients from productive processes, directly reduce productive processes, alter disease resistance, and increase individual variation in productive processes.

Environmental Design. Design of environmental modifications is based on many considerations: the cost/benefit ratios of available alternatives; the elaborateness of the facility and its environmental-regulatory mechanisms; providing special facilities for handling animals when being examined or medicated and when being marketed; and in all facilities, once the animals' requirements have been met, accommodating the workers' needs. Primary modifications are such things as shades, windbreaks, and sprinklers, intended to protect the animals against extremes. Secondary environmental modifications, such as closed houses, provide more environmental control and aim to more nearly fulfill the animals' environmental requirements by means of insulation, ventilation, temperature and humidity control, lighting, flooring, and other equipment.

Environmental Management. The range of production environments employed successfully in U. S. animal agriculture today--from the open range to a single-animal pen in a closed house--call for vastly different kinds and amounts of management. Various production schemes have been designed with these differences in mind. But a system that is economically feasible today will not necessarily be so in the future. The basic assumptions tend to change, consequently the relative feasibilities of the production systems change, also. Pertinent public policy issues between the present and the year 2000 in regard to environment and behavior include: energy resources--grain and forage production/processing/transportation, facility construction/operation, animal transportation/marketing/processing; labor resources--availability/location of workers; and public concerns--environmental protection, well-being of animals in intensive systems, well-being of predators in extensive systems.

Major Lines of Research

New technologies in applied animal-agricultural ecology are likely to emerge as the result of currently ongoing research by 2000. They most likely will occur along four lines: energy conservation, optimization of stress, stress and immunity, and photoregulation of physiological phenomena.

Energy Conservation. Feed and fuel--sources of energy--account for much of the cost of animal production. Decisions pertaining to energy economics will determine the future of U. S. animal agriculture. Today, few swine and poultry and few finishing lambs and cattle forage for themselves; their food is harvested, processed, and transported to them. In recent decades, animal production has grown in the Sun Belt, where less insulation and heating are required, and waned in the Corn Belt, where more feedstuffs are produced. Results of broad ongoing research and development efforts focusing on the total energy cost of animal production will soon be implemented in the animal industries so economies can be realized in terms of joules and dollars.

Optimizing Total Stress. Life entails stress inexorably. It is well-known that environmental stressors affect animal performance, both directly and indirectly. Heretofore, stress-management strategies have been aimed at eliminating or at least minimizing negative impacts of environmental stressors. As more has been learned about the complexities of both environmental stresses and animal perceptions and responses, it has become clear that a more rational approach would be to optimize the quality and quantity of stress to which an animal is subjected; either too much or too little environmental stimulation can have deleterious effects on the performance, health, and overall well-being of agricultural animals. In order to be able to optimize total stress, more must be learned not only about how individual stressors act on, are perceived by, and are responded to by animals, when they occur singly, but also--because animals usually face more than one stressor--about how stressors interact sequentially and simultaneously. Results of research now in progress should make it possible to fine-tune artificial environments more closely to more nearly meet the animals' needs. Computerization of animal- and environment-sensing devices and environmental-control equipment will make biofeedback-linked automation of environmental regulation a reality in animal agriculture. Specific kinds of operant technology, in which the animals operate certain environmental-control devices to suit themselves, are already being recommended to farmers.

Stress and Immunity. Intensification of animal production has been accompanied by new challenges in disease management. Animals function better when living in harmonious balance with the other living forms and the physical and chemical factors in the surroundings. The problems of stress-altered disease resistance must be dealt with continually. A variety of common environmental stressors--cold and hot environments, crowding, mixing, weaning, limit-feeding, noise, and movement restraint--are known to alter animals' defenses against infectious agents. Still, unequivocal quantitative evidence for specific infectious diseases is difficult to obtain. Indeed, little evidence is yet available, because the phenomena involve relations among complex entities--environment, immunity, and infectious agents. Normal regulation of the immune system is an active, dynamic process, controlled by various lymphoid cells which interact with one another and either enhance or inhibit various antigen-specific immune processes. It has been proposed that the function, differentiation, or perhaps the number of regulatory lymphoid-cell subsets is affected differentially by environmental stressors. Certain adaptation hormones alter specific functional immune events at physiological concentrations in vitro. Also, the relative importances of respective lymphoid-cell subsets depends on the nature of the pathogenic insult present. Basic scientific techniques developed in recent years--coupled with more traditional neurobiological, endocrinological, and immunological approaches--hold much promise for yielding in the near future a better understanding of how stressors influence regulatory signals among lymphoid-cell subpopulations and thus how the environment influences specific immunological traits.

Photoregulation of Physiological Phenomena. The use of photoperiod management has revolutionized the poultry industry. The light environment is managed in a relatively simple and inexpensive way so as to enhance a stimulatory effect on the poultry rather than minimize an inhibitory effect. Ungulates appear to be less photoreactive than birds, but nonetheless they do respond. Photic influences on the sheep's reproductive functions have been well-characterized in the last two decades, and a few producers already manage the photoperiod in sheep environments. More recently, effects of the light environment on reproduction, lactation, and even growth have been demonstrated in cattle and swine. There is considerable interest in gaining a more thorough understanding of these relationships in the ungulates so general recommendations to producers can be formulated.

CHAPTER 2

CURRENT STATE OF THE ART

Energy Conservation. Basic principles of biological and physical energy flows and interconversions in agriculture are understood reasonably well, and tradeoffs between feed and fuel have been quantified for most species. There remains to be elucidated, however, numerous details which, when taken together, will enable additional energy savings in animal production systems. A few examples will demonstrate the range of pertinent areas.

Environmental-temperature cycles and adjustments. Environmental-temperature management schemes developed during an era of cheaper energy are too luxurious. For example, there has been a tendency to maintain constant environmental temperatures even though the animals evolved in a thermal environment that fluctuated cyclically. It has been found that (a) young pigs given the opportunity to regulate environmental temperature for themselves inserted a 20 F^o daily cycle of warm afternoons and cool nights, (b) this 20^o cycle resulted in a 50-percent reduction in fuel use during cold weather, and (c) an artificially cycling environment similarly resulted in unchanged pig performance and a 30-percent fuel savings. Timely downward adjustments of thermostats that parallel age-dependent changes in thermal requirements also save fuel. A bonus in some cases is that cooler surroundings spur appetites, so performance actually increases.

Heat exchange and solar-energy collection/storage. Operating principles of various kinds of heat exchangers and solar heating systems are well-known. Cost-effective, low-maintenance designs being developed in the private sector will be readily adopted by the animal industries when they reach the market.

Energy self-sufficient systems. The systems approach is being taken to integrating energy use in animal production operations. The "energy farm" at the University of Nebraska is one of several attempts in the United States to develop agricultural systems that approach energy self-sufficiency.

Optimizing Total Stress. Basic knowledge of environmental stress and animal responses is meager. It will be possible to optimize stress in animal agriculture only after much more is learned about these complex phenomena.

Multiple simultaneous stressors. Several fundamental questions need to be answered. When two or more stressors act at the same time, their combined effect might equal the sum of their respective effects when acting alone, or it might be more or less than that sum. The ways stressors combine and the ways animals integrate and compensate for multiple stressors are just now being investigated.

Perception of stress. The limits of experiences which give animals pleasure or displeasure, comfort or discomfort, pain or the absence thereof, remain enigmas. An animal's perception of a stressor apparently depends not only on the intensity and duration of the environmental impingement, but on the animal's psychological and physiological states. Furthermore, experimental lines of chickens have been developed through genetic selection for their perception of social stress. Consider these additional pieces of evidence. (a) When the environment is more controllable or at least more predictable, animals tend to feel more comfortable, even when responding to stressors. On the other hand, when the environment is neither predictable nor controllable, animals sometimes become frightened or anxious, and eventually they might assume the state of helplessness and suffer its various consequences. (b) Alliesthesia is the phenomenon via which an animal's bodily status affects its perception of the environment. Hence, a given environment is not necessarily perceived consistently by a given animal. (c) Some environments contain features that give animals the opportunity to cope (alleviate stress) by engaging in adjunctive or substitute activities--such as play--when they are frustrated, have unfulfilled expectations, or lack control of their surroundings; other environments do not. (d) Some animals given the opportunity to control environmental temperature or illumination level, for example, purposefully inject cycles into their surroundings, suggesting that they prefer environmental cycles. Influences such as these few just mentioned make it all the more difficult to identify the complex of environmental conditions that would ensure a sense of well-being in a specific animal at a particular time. They further suggest that operant technology might have advantages; the sense of control an operantly conditioned animal presumably possesses very well might enhance the animal's sense of well-being. In any event, when it becomes possible to arrange tests in which animals can accurately express their preferences for important environmental features, the resultant information will be used immediately in the programming of environmental-control systems.

Stress and Immunity. The regulation of immune phenomena sometimes takes the form of collaboration between T- and B-cells. It is known, for example, that separate T-cell subpopulations play helper and suppressor roles, respectively, in regard to antigenic stimulation of certain B-lymphocytes. Several other relations have been characterized, too. Environmental stressors seem to act at several points of interaction among lymphoid cell subpopulations. For example, results of recent experiments suggest that: (a) weaning pigs younger than five weeks of age impairs T-cell-mediated immune responses, (b) a slight but significant reduction in B-cell-mediated synthesis of antigen-specific antibody occurs when young pigs are injected with antigen at weaning time, and (c) effects of cold stress and weaning on antibody synthesis are additive. It appears to be most likely that hormonal influences lie at the center of these relationships. If the general notion that hormones affect the differentiation and function of lymphoid cells eventually is proven to be correct, then it will still remain to be explained how different components of the immune system can respond differentially to the same stressor, how the same component can respond differentially to different stressors, and how the same components can respond differentially to the same stressor at different times. When the basic phenomena involved in stress effects on immunity are understood, this information, in conjunction with better knowledge of stress per se that is being developed, can be used to optimize stress in terms of both performance and immunity.

Photoregulation of Physiological Phenomena. Photocontrol of reproduction in female and male birds is understood well in both its basic and applied aspects, and this knowledge has been applied in the poultry industries. Of the ungulates, the ewe and ram have been studied most thoroughly in this connection. Results of similar studies of cattle and swine have been less clear, probably because the basic relations between these animals and their photic environment have not yet been established. Nevertheless, some results have been encouraging: under controlled lighting, sows weaned heavier piglets, cows yielded more milk, and lambs grew faster. Experiments now in progress hold much promise for producing information which will be immediately applicable in animal production.

CHAPTER 3

MAJOR BREAKTHROUGHS NEEDED, INSTITUTIONAL ARRANGEMENTS, AND LIKELY TIMING

Energy Conservation

Major Breakthroughs Needed

The energy conservation thrust needs major breakthroughs in:

(a) the successful integration of available information from biology and engineering into feasible systems,

(b) the development of cost-effective designs of heat exchangers and solar heating systems, and

(c) basic information on animals' requirements for and tolerances of environmental cycles.

Funding. Area (a)--a tripling of research support might cut time to adoption in half. Area (b)--proceeding at acceptable pace. Area (c)--needs much more emphasis; e. g., $1 million/year.

Institutional Arrangements

The groups engaged in research and development in these respective areas will be primarily:

(a) federal agencies and state agricultural experiment stations,

(b) private industrial firms, and

(c) state agricultural experiment stations.

Likely Timing

The appearance of significant new technology adoptable in agricultural practice assuming continuing research support at present levels would come: (a) by 2000, (b) by 1990, and (c) by 1990.

Optimizing Total Stress

Major Breakthroughs Needed

Before necessary applied research can be conducted, more basic knowledge of stress perception and responses by agricultural animals must be generated. These investigations would focus on both physiological and behavioral adaptations.

Funding. Research support of at least $5 million/year for a period of ten years would be needed if significant progress is to be made.

Institutional Arrangements

State agricultural experiment stations and Federal agencies would be involved in increased research activity in this area.

Likely Timing

If increased research support began now, significant new information would emerge by 1990. Fundamental changes in animal production systems would require feasibility studies in the 1990s, and likely adoption of this new technology would not occur before 2000.

Stress and Immunity

Major Breakthroughs Needed

Development of more knowledge of stress effects on immune events is now hampered by the paucity of basic information on immune phenomena. Breakthroughs in this area will necessarily precede complete development of management strategies aimed at optimizing stress level in terms of immune status. At the same time, research on the fundamental relations between the hormones of adaptation and immune processes should be increased markedly to take advantage of basic information as quickly as possible.

Funding. Basic immunological research is funded at a relatively high level. Research on stress and immunity must be supported at a higher level--e. g., $5 million/year for ten years--if significant progress is to be made.

Institutional Arrangements

The basic immunological research will be conducted for the most part at Federal, state, and private biomedical research laboratories. Effects of stress on the immunity of agricultural animals will be studied in state agricultural experiment stations.

Likely Timing

New basic technology will emerge in this decade. New technology regarding stress and immunity will probably emerge during the 1990s and be adoptable by 2000.

Photoregulation of Physiological Phenomena

Major Breakthroughs Needed

Basic knowledge of agricultural ungulates' requirements for light in terms of photoperiod, spectral quality, and luminous intensity is needed before marked progress can be made in learning how to use light management in livestock production.

Funding. More funding is needed to support basic research in this area--e. g., $1 million/year for ten years.

Institutional Arrangements

Research on ungulates' responses to photic elements of the environment will take place at state agricultural experiment stations and Federal agencies.

Likely Timing

Because the basic frameworks have been constructed in the poultry industries, development of readily adoptable technologies should proceed relatively rapidly. New technologies should emerge by 1990.

CHAPTER 4

POTENTIAL PRIMARY IMPACTS

Energy Conservation

Developments in this area will determine the overall structure of animal agriculture in the United States. Its effects will be profound--from the farmer to the consumer to the national economy. Systems compatible with current farming approaches will be developed, so retrofitting/renovating facilities and implementing changes in management schemes can proceed with minimal disruption of ongoing activities.

In view of continuing energy concerns, these technologies will be adopted readily by farmers. Capital-investment requirements will be moderate. All animal-agricultural industries will be involved.

Optimizing Total Stress

The output of these new technologies will be the development of management practices that will increase animal well-being and performance. These practices will mostly take the form of new environmental-control systems and programs, but some new equipment will emerge, too. Farmers will adopt these technologies at only a moderate pace--often at the time of constructing new facilities, for example--because the benefits often will be subtle. Moderate capital investments will be required. The swine and poultry industries will be most involved in this area.

Stress and Immunity

Technologies in this area would include management programs aimed at optimizing stress levels with health prevention and control. Farmers would adopt the new information at a moderate rate. Little capital investment would be required apart from physical changes in facilities aimed at optimizing stress level. All animal industries would be involved.

Photoregulation of Physiological Phenomena

Technologies in this area have been developed and adopted widely in the poultry industries. They will likewise be adopted rapidly in the livestock and dairy industries when they become available. They are simply and inexpensively implemented in intensive production systems. The benefits would include improved reproductive efficiency and productive performance.

CHAPTER 5

REFERENCES

Baldwin, B. A., and Meese, G. B., "Sensory Reinforcement and Illumination Preference in the Domesticated Pig." Anim. Behav. 25:497-507, 1977.

Blecha, F., and Kelley, K. W., "Effects of Cold and Weaning Stressors on the Antibody-Mediated Immune Response of Pigs," J. Anim. Sci. 53:439-447, 1981.

Blecha, F., Pollman, D. S., and Nichols, D. A., "Weaning Pigs at an Early Age Decreases Cellular Immunity," J. Anim. Sci. 56:396-400, 1983.

Bruce, J. M., "Ventilation and Temperature Control Criteria for Pigs," in Environmental Aspects of Housing for Animal Production, J. A. Clark, ed. (Boston: Butterworths, 1981).

Cabanac, M., "Thermoregulatory Behavior," in Environmental Physiology, D. Robertshaw, ed. (Boston: Butterworths, 1974).

Curtis, S. E., "Pig Energetics--The Diet and the Environment," Proc. Pork Industry Conf., 1981, Champaign, Ill., 93-105 (Urbana, Ill.: Univ. of Ill. at Urbana-Champaign, 1981).

Curtis, S. E., Environmental Management in Animal Agriculture, (Ames, Ia.: Iowa State Univ. Press, 1983).

Curtis, S. E., "Perception of Thermal Comfort by Farm Animals," in Farm Animal Housing and Welfare, S. H. Baxter, M. R. Baxter, and J. A. C. MacCormack, eds. (Boston: Martinus Nijhoff, 1983).

Curtis, S. E., "What Constitutes Animal Well-Being?," in Animal Stress: New Directions in Defining and Evaluating Effects of Stress, G. P. Moberg, ed. (Bethesda, Md.: American Physiological Society, in press, 1984).

Curtis, S. E., and Kelley, K. W., "Environment and Health in the Hog House," Proc. Pork Industry Conf., 1983, Champaign, Ill., 56-72 (Urbana, Ill.: Univ. of Ill. at Urbana-Champaign, 1983).

Curtis, S. E., and Morris, G. L., "Operant Supplemental Heat in Swine Nurseries," Proc. Int. Livestock Environment Symp., 2nd, Ames, Ia., 295-297 (St. Joseph, Mich.: American Society of Agricultural Engineers, 1982).

Dantzer, R., and Mormede, P., "Pituitary-Adrenal Consequences of Adjunctive Activities in Pigs," Horm. Behav. 15:386-395, 1981.

Dawkins, M. R., "Battry Hens Name Their Price: Consumer Demand Theory and the Measurement of Ethological Needs," Anim. Behav. 31:1195-1205, 1983.

Duncan, I. J. H., "Animal Rights--Animal Welfare: A Scientist's Assessment," Poul. Sci. 60:489-499, 1981.

Duncan, I. J. H., and Hughes, B. O., "Free and Operant Feeding in Domestic Fowls," Anim. Behav. 20:775-777, 1972.

Goetsch, W. D., and Muehling, A. J., "Evaluation of Earth-Tube Heat Exchangers as Air Tempering Systems for Swine Farrow-Nursery Housing," Paper 83-4073 (St. Joseph, Mich.: American Society of Agricultural Engineers, 1983).

Holmes, C. W., and Close, W. H., "The Influence of Climatic Variables on Energy Metabolism and Associated Aspects of Productivity in the Pig," in Nutrition and the Climatic Environment, W. Haresign, H. Swan, and D. Lewis, eds. (Boston: Butterworths, 1977).

Kelley, K. W., "Stress and Immune Function: A Bibliographic Review," Ann. Vet. Res. 11:445-478, 1980.

Kelley, K. W., "Environmental Effects on the Immune System of Pigs," Pig News and Inform. 3:395-400, 1982.

Kelley, K. W., "Immunological Consequences of Changing Environmental Stimuli," in Animal Stress: New Directions in Defining and Evaluating Effects of Stress, G. P. Moberg, ed. (Bethesda, Md.: American Physiological Society, in press, 1984).

Kilgour, R., Foster, T. M., and Temple, W., "Operant Technology Applied to Solving Farm Animal Problems," paper presented at the meeting of the New Zealand Psychological Society, Hamilton, N. Z., August 1982.

Midwest Plan Service, Solar Livestock Housing Handbook (Ames, Ia.: Midwest Plan Service, 1983).

Muehling, A. J., and D. D. Jones, "Livestock Producer Needs for Improving Production Efficiency," paper presented at the 1983 Winter Meeting of the American Society of Agricultural Engineers, Chicago, Ill., December 1983.

National Research Council, Effect of Environment on Nutrient Requirements of Domestic Animals (Washington, D. C.: National Academy Press, 1981).

Price, E. O., "Behavioral Aspects of Animal Domestication," Q. Rev. Biol. 59:1-32, 1984.

Puckett, H. B., Olver, E. F., Harshbarger, K. E., and Spahr, S. L., "Automating the Eyes of the Herdsman," J. Anim. Sci. 53:516-523, 1981.

Petitclerc, D., Chapin, L. T., Emery, R. S., and Tucker, H. A., "Body Growth, Growth Hormone, Prolactin and Puberty Response to Photoperiod and Plane of Nutrition in Holstein Heifers," J. Anim. Sci. 57:892-898, 1983.

Schulte, D. D., and Splinter, W. E., "Energy-Integrated Farming: Research and Demonstration for Energy Self-Sufficiency," Farm, Ranch and Home Quarterly 30:6-13, 1983 (Lincoln, Neb.: Univ. of Neb.)

Shelton, D. P., and Brumm. M. C., "Reduced Nocturnal Room Temperatures in a Flat-Deck Swine Nursery," Paper MCR 84-124 (St. Joseph, Mich.: American Society of Agricultural Engineers, 1984).

Spahr, S. L., "Extending the Capability of the Livestock Manager Through Electronics," paper presented at the 1983 Winter Meeting of the American Society of Agricultural Engineers, Chicago, Ill., December 1983.

Stevenson, J. S., Pollman, D. S., Davis, D. L., and Murphy, J. P., "Influence of Supplemental Light on Sow Performance During and After Lactation," J. Anim. Sci. 56:1282-1286, 1983.

Stricklin, W. R., ed., Proc. Md. Conf. on Animal Behavior in Agriculture, 1982, Port Deposit, Md., Appl. Anim. Ethol. 11:295-429, 1984.

Tucker, H. A., and Ringer, R. K., "Controlled Photoperiodic Environments for Food Animals," Science 216:1381-1386, 1982.

Wiepkema, P. R., "On the Identity and Significance of Disturbed Behaviour in Vertebrates," in Disturbed Behaviour in Farm Animals, W. Bessei, ed. (Stuttgart: Eugen Ulmer, 1982).

Background Paper No. 8

Crop Residue and Animal Waste Use

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UTILIZATION OF CROP RESIDUES AND ANIMAL WASTES

Summary

Improved use of crop residues and animal wastes represents a tremendous potential for more efficient use of resources. Livestock on U.S. farms produce about 55 million tons of recoverable manure. Approximately 363 million tons of residues are recoverable from cash grain production. Several technologies and major lines of research and development exist in this area: (1) energy from manure, (2) animal feed from manure, (3) chemicals from crop residues, and (4) animal feeds from crop residues.

The high volume of manure production that occurs at many large feedlots and dairies is an opportunity in disguise. Manure has value both as a soil additive and as a source of energy for heat and electricity. Traditionally, manure has been either applied to the soil surface in an unprocessed form or disposed of in a sewage lagoon. Application of manure to the soil surface creates environmental problems in many areas and results in a loss of up to 90 percent of the useful nitrogen value of the manure. Technology is available to inject the manure below the soil surface, resulting in only a five-percent loss of nitrogen.[1]

Large farms may benefit from installing anaerobic digestors to produce methane from manure for use as a heating fuel or as a substitute for propane in electric generators. The slurry that remains after digestion contains most of the original nutrient value and may be applied to cropland as fertilizer. Injection of the slurry is preferred since most of the nitrogen is in the form of ammonia after digestion.

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[1] Suttan, A. L. et al., "Utilization of Animal Waste as Fertilizer," Purdue University, Cooperative Extension Service, Miner, ID, 101, 1975.

In many farm operations, it is profitable to process manure and use it as a source of nonprotein nitrogen and fiber in cattle and dairy cow rations. Manure is a low-cost source of nutrients and reusing it as feed reduces the volume of animal wastes that must be processed or disposed of. If used for cattle feed, manure must first be concentrated, then processed by heat treatment or by ensiling.

Using crop residues as a source of chemical feedstocks and animal feed involves some complex tradeoffs in most areas because crop residues are becoming widely valued for their ability to reduce soil erosion in combination with conservation tillage practices. Even when crop residues are completely tilled into the soil, they have significant value in maintaining soil structure and nutrient content. However, useful amounts of residues may be removed from fields in many parts of the United States where cropland slopes are gentle and residue density is high.

The cost of transporting bulky crop residues generally constrains the area over which collection is economically feasible.

Several technologies under development have promise in areas where residue collection is economically feasible. Residues may be broken down into their component parts by mechanical, chemical, or biological processing, or a combination of all three. The principal components of crop residues are lignin, hemicellulose, and cellulose. Lignin can be used to produce solvents such as benzene, toluene, and xylene. Hemicellulose is readily converted into furfural, which is, in turn, a feedstock for the production of numerous chemicals. Plastic films and fibers and the simple sugar, glucose, can be produced from cellulose. Production of these chemicals is likely to require moderately large-scale technology based on industrial processes and equipment.

Transportation costs reduce the likelihood that crop residues will be used as feedstock for industrial processing. Some farms may adopt direct combustion of crop residues for use as a source of heat for grain drying.

In the near term, the most likely process for conversion of crop residues is biological: ruminant animals. Most crop residues can be fed directly to ruminant animals as a source of roughage. A substantial potential exists for development of technologies to increase the palatability and digestibility of crop residues. Numerous efforts have been made to develop simple mechanical and chemical pretreatments, with some success. The problem is difficult, owing to the degree with which the digestible hemicellulose and cellulose are bound to the nondigestible lignin component in the residues of mature cash grain crops. Additional research and development leading to economic and effective pretreatments would have substantial benefits, since the size of this resource is so large.

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TABLE 1. Crude protein, total digestible nutrients and digestible energy values for selected crop residues.

INTRODUCTION

Agricultural production is a renewable resource based on a nonrenewable resource--LAND. In the United States, of the 1.36 billion acres of nonfederal land devoted to agriculture, approximately 30% is used for crop production, 30% is in rangeland, 28% is in forest and 12% is pasture. Each year, approximately 3 million acres are removed from this land base to supply the demands for houses, highways, airports, etc., for an increasing population. Approximately 126 million acres of medium potential land are available in reserve to offset this loss, but 59 million of these acres will be required for the expanding population by the year 2000. Considering the projection on crop yield improvements and farming efficiency, the remaining 67 million acres will not be adequate to supply food for domestic consumption and exports (Kenney, 1983). The land that is presently being utilized can be made more efficient through utilization of byproducts of the present agricultural production system. For example, for each pound of corn grain produced, there is also approximately one pound of corn stalks and cobs produced. For wheat and soybeans, the ratio is approximately one pound of grain for one and one half pounds of residue. In a swine operation, for each pound of pork produced, there is also 8 pounds of dry manure produced.

Definition and Scope

This report will discuss the present and future utilization of crop and animal residues and their impact on increasing the efficiency of agricultural land use. For the purposes of this report, crop residues will be defined as the residual plant dry matter remaining after removal of the primary product for which the plant was grown while animal waste is defined as fecal, urine and wastage from an animal production system. These definitions should be distinguished from biomass which is all material resulting from photosynthetic action. The term biomass includes crop residues and animal waste as well as all plant matter. The emphasis of this report will include the direct utilization and pretreatment and processing techniques for converting crop residues and (or) animal waste into fertilizer, feed, fuel, fiber and chemicals.

Major Lines of Research

Several residue and waste utilization systems are being researched which could reach commercial development by the year 2000. These systems can be categorized into four major areas: energy from manure; chemicals from crop residues; animal feed from crop residues; and animal feed from manure. Each of these conversion techniques will be discussed and a specific example of each will be given.

Energy from Manure. Conversion of animal manure to methane gas via anaerobic fermentation will be implemented on farms because of the trend toward larger production units, manure disposal problems and the increasing cost of the energy required to produce animals in these facilities. Raising animals in an environmentally controlled facility increases the electrical and thermal energy demands as well as the manure handling and disposal requirements. Anaerobic fermentation of the manure produces methane gas which will fuel an internal combustion engine coupled to an electrical generator. The thermal energy from the engine and the electrical energy from the generator are used in the livestock facility for environmental modifications, feed processing and product handling. Excess electricity can be sold to the local electric utility (Public Utilities Regulatory Policy Act, 1978).

Chemically Processing Crop Residues. Before the advent of inexpensive petroleum products, the raw materials from which the organic chemicals were produced came from wood, animal and plant products and coal. When the petroleum industry developed, it replaced the solvents that were originally obtained from fermentation synthetic films and fibers replaced wool, cotton, plant and animal products. As the cost of crude oil began to increase, chemicals from biomass products became more economical. One hundred and ninety five billion pounds of chemicals (excluding fuels) are produced each year from nonrenewable resources. An additional 30 billion pounds of chemicals are produced from carbohydrates and other organic sources (Dunlap, 1983). The first step in utilizing crop residue for chemical production is to increase the efficiency of converting a residue into a usable chemical such as glucose. Presently, the efficiency of converting a lignocellulose crop residue to glucose is less than 50%. Recently, conversion techniques such as the ammonia freeze explosion technique (Dale, 1983) have been shown to increase this efficiency to 90%. Once the conversion efficiency increases, the economic environment will dictate whether the end products will be fuel, food or chemicals.

Animal Feed from Crop Residue. In addition to engineering chemicals through biological processes from crop residues, the residue will also be treated and used as a component in diets for ruminant animals (sheep and cattle). Ruminant animals are converters of low quality residues into protein rich, nutrient dense, human food. One method of increasing the low digestibility of crop residues for ruminant animals is to treat the residue with anhydrous ammonia in a sealed environment. This process not only increases the protein content of the residue but also increases residue digestibility (20%) and intake (15%) (Paterson, 1981).

Animal Feed from Manure. The nutrients and minerals in livestock manure can be processed and formulated into diets for animals. Rations supplemented with animal manures have been shown to give equal production performance compared with diets supplemented with plant grain protein. Poultry litter has been ensiled and fed successfully to ruminant animals for many years (Vestal, 1984). Recently, more innovative developments have occurred which have combined the effluent from an anaerobic digester system with a crop residue to produce an ensiled diet. This increased the protein value of the silage by three to four percent (Goedeken, 1984). In animal production systems, innovative total recycling systems will be developed to more completely utilize the nutrients before discharging them into the environment. Hopefully this approach will reduce production costs.

CURRENT STATE OF THE ART

A farmer does not consider crop residues and animal waste in the same context. Crop residues can be a problem because a farmer has to deal with them in order to prepare a seedbed for the next crop, whereas animal manure is material which the farmer is required by law (Environmental Protection Agency) to dispose of in a manner which does not pollute the environment. Therefore, the State of the Art of crop residue utilization and animal waste utilization will be discussed separately. Each discussion will be subdivided into direct utilization and pretreatment and processing utilization.

Crop Residues

The quantity of crop residues produced annually in the United States is approximately 363 million tons. The quantity of residue produced is directly related to the amount of grain produced by the plant. For example, on a pound-to-pound basis, the ratio of residue to grain for corn is one-to-one. For wheat and soybeans, the ratio is one pound of grain to one and one half pounds of residue (Larson, 1978). The permissible amount of residue which can be removed from the soil will depend on several factors including climate (temperature and moisture), inherent soil properties, topography, fertilizer added, crops planted and soil and residue management systems.

Direct Utilization. First and foremost, the crop residue is used to prevent soil erosion. Research has shown that conservation tillage which leaves approximately 3000 pounds/acre of residue on the surface, reduces soil loss by 50% throughout southern Minnesota and Iowa when compared with conventional tillage which leaves no residue on the surface (Larson, 1978). The crop residue also contains some of the nutrients required for production of another crop the following year. An estimate of the amount of the nitrogen, phosphate and potassium contained in the residue from nine leading crops in the United States is 40%, 10%, and 80%, respectively, of the nutrients applied to produce the crop. After the erosion protection and tilth requirements of the soil are met, the excess residue can then be removed. The quantity of residue that can be removed depends on several factors as previously stated. In some instances, it is to the advantage of the farmer to remove some of the crop residue in order that a seedbed can more easily be prepared for the next crop.

In the animal industry, crop residues are used either as bedding or as a roughage source in animal diets. The feeding value of these residues varies with the species and maturity of the plant. A comparison of several common residues to corn silage shows a wide range of crude protein (2.5 to 9.5%), total digestible nutrients (42 to 68%) and digestible energy (1.99 to 2.98 m cal/kg; table 1; National Academy of Sciences, 1971). Increasing the amount of available nutrients from crop residues for ruminant animals can be accomplished by chemical pretreatment and (or) physical processing. Emphasis should be placed on increasing residue intake and nutrient digestibility by the ruminant animal.

Pretreatment and Processing. Crop residues are primarily composed of lignocellulosic material which is chemically composed of lignin, hemicellulose and cellulose. The percentage of these components in a residue varies with both plant species and maturity. For most crop residues, the percentage of lignin

TABLE 1

CRUDE PROTEIN, TDN AND DIGESTIBLE ENERGY VALUES FOR SELECTED CROP RESIDUES[a]

Item	Crude protein %	Total digestible nutrients (TDN) % (cattle)	Digestible energy mcal/kg (cattle)
Corn Silage	9.5	67.6	2.98
Corn Stalks	6.8	60.9	2.68
Soybean Straw	5.2	42.1	1.86
Rice Straw	4.5	45.2	1.99
Wheat Straw	3.7	48.8	2.15
Corn Cobs	2.5	48.2	2.13
Fescue Hay	6.0	53.2	2.35

[a] Dry matter basis.

Taken from National Academy of Sciences. 1971. Washington, D.C.

ranges from 10 to 20%, hemicellulose from 20 to 30% and cellulose from 40 to 60%. Lignin in forage crops can contain as much as 2% nitrogen which is normally unavailable to the animal for anabolism.

Lignin is a non-carbohydrate indigestible portion of the plant which give the plant structural strength. Unfortunately from a nutritional standpoint it is interwoven with the digestible carbohydrates; cellulose and hemicellulose. The lignin is not only resistant to microbiol degradation, but it also retards the extent of digestion of the cellulose and hemicellulose fractions (Van Soest, 1982). Since hemicellulose may be bound directly to the lignin, its extent of utilization in biological systems is dependent on the degree of liquification. Cellulose occurs with the lignin, but in a complex which decreases cellulose digestibility as lignin concentration increases. Thus, to increase the efficiency of converting a crop residue into useful chemicals via a biological process, the hemicellulose and cellulose must be disassociated from the lignin.

Numerous methods of treating lignocellulose materials are being investigated (Hawley, 1983; McCarty, 1983). Basically all of these methods use one or all of the following; disrupt the crystalline structure of the cellulose; disassociate the lignin from the cellulose and hemicellulose fractions or decrease the particle size of the material in order to increase reactive surface area. The methods of pretreatment include adding chemicals to the residues (e.g. acids, alkalis, salts) or biologically pretreating the residue with enzymes. These pretreatment methods increase the availability of cellulose and hemicellulose but do not destroy lignin. Lignin can be removed from the residue by further chemical treatment or biologically by the use of aerobic bacteria or fungi. The chemical method produces very dilute solutions which are expensive to concentrate. The biological method requires weeks and perhaps months to be effective and also has the disadvantage of degrading the cellulose and hemicellulose fractions.

Instead of attempting biological conversion of lignocellulose residue, it could be chemically divided into its three components; lignin, hemicellulose and cellulose. Each component would provide basic chemicals useful to man. From the lignin component, solvents such as benzene, toluene and xylene could be produced. From the hemicellulose component, furfural (which is the raw material necessary for the production of numerous chemicals) could be manufactured. Films, fibers and glucose could also be produced from the cellulose.

Although chemical processing of the lignocellulose residue provides many possibilities, biological processing will likely occur in the future for several reasons. First, if a biomass resource is to be used for chemicals, it will be grown solely for that purpose and optimized for specific chemicals. Secondly, crop residues are dispersed geographically and have low bulk densities. Therefore, long distance transportation of large quantities of residues for chemical processing is probably not economically feasible.

In the near term, the most likely biological process for converting of crop residue into a useful product is via the ruminant animal. New methods for improving digestibility and intake of residues are needed. Other biological processes such as fermentation for liquid or gaseous fuels could significantly impact energy supplies (especially the farm sector) as the reserve of petroleum products continues to decrease (U.S. Geological Survey, 1983).

Crop residues are being evaluated as sources of thermal energy by direct combustion, gasification, or pyrolysis for on-farm energy use. Innovative concepts such as utilizing corn cobs to dry the corn grain are being developed (Liljedahl, 1983; Kline, 1983; Morey, 1983). Processes such as this will likely occur on the farmstead because of economics of transporting residues. Direct combustion of residue destroys all organic matter with only the ash (containing a low concentration of minerals) remaining for recycling to the land.

Animal Waste

Of the 112 million tons of manure voided by farm animals in the United States each year, approximately 50% is economically recoverable (Gilbertson, 1978). The manure that is economically recoverable has to be properly disposed of in order to abate any pollution. The trend has been to dispose of the manure in lagoons or other treatment facilities. However, with increasing energy and fertilizer costs, the minerals and protein value of manure are being utilized.

Direct Utilization. The quantity of manure produced from dairy beef, swine and poultry confinement operations could supply 16, 30 and 40% of the total nitrogen, phosphorous, and potassium required for crop fertilization (Prior, 1975). In addition to these major nutrients, micronutrients are also supplied by the manure. The organic matter in manure can improve the tilth of the seedbed, seedling emergence and root penetration (Wilkerson, 1979). Recommendations for manure applications on the soil are site specific and depend on the type of soil, salt content of the manure and amount of soil leaching (Steward, 1974). Method of manure application is positively correlated with amount of nitrogen applied to the soil. Nitrogen is easily lost via ammonia volatilization, with the loss as high as 90%. In a study reported by Sutton (1975) 27% of the total nitrogen applied was lost within 4 days of application when manure was broadcast on the land. Injecting manure into the soil resulted in only a 5% loss. Since land is not always available for manure application, storage structures that can accumulate the manure during the off-season must be built. Major losses of nutrients can occur during storage and are primarily dependent upon storage conditions. As much as 90% of the total nitrogen may be lost with lagoon storage (Vanderholm, 1975).

Feeding animal manures reduces manure disposal problems, feed costs and recycles nutrients. Nutrient value of manure varies with livestock species, type of diet fed to livestock and handling and storage of the manure. Poultry manure is generally more digestible than cattle manure with swine manure being intermediate. Of the 5.1 billion pounds per year of collectible nitrogen excreted by livestock in the United States, only 22% is in the form of amino acid nitrogen which can be directly utilized by monogastric animals (Smith, 1973). The energy value of livestock manure is low. The estimated total digestible nutrient value for broiler, layer, steer, and cow waste is 59.8, 52.3, 48 and 45%, respectively ((Fontenot et al., 1983). In addition, cattle manure can contain up to 71% crude fiber (cell walls) which reduces digestibility when used in swine and poultry diets. Therefore, since the ruminant animal can efficiently utilize nonprotein nitrogen and crude fiber it is the most efficient target animal for rations which incorporate manure.

Pretreatment and Processing. Treatment of livestock manure is desirable in order to prevent potential disease problems when refeeding (Fontenot and Webb, 1975). Heat, chemical and ensiling treatments are methods of reducing pathogenic activity in animal manure. Ensiling manure alone or with other feedstuffs results in generation of moderate heat and a reduction in pH which may offer a more economical alternative than sterilization by costly chemical or heat treatment (Fontenot and Webb, 1975). Complete dehydration or partial drying of manure can reduce nutrient availability and for this reason may not be recommended (Taylor, 1974). Animal digestibility of manure which is high in fiber can be increased by treatment with caustic bases (Smith et al., 1969). Potential harmful drugs, pesticides and minerals have been detected in livestock manures. However, there is no documented evidence that recycling of animal manure presents hazards to human health. Refeeding of manure has not affected the taste of meat, milk or eggs, nor has animal health or production efficiency been impaired.

Pretreatment of animal manure is usually confined to solids separation. Various techniques are used to separate solids such as settling, centrifuging and screening. Commercial screening and centrifuging equipment is available to recycle solids back into animal diets. An example of this would be in a farrow-to-finish swine operation in which the solids from the finishing building are collected, concentrated and refed to the sows in the gestation building.

Processing of animal manure generally involves a fermentation process. Ensiling manure for production of feed material is popular. However, research devoted to producing single cell protein from nutrient waste streams and producing algae from animal manures has been limited. Production of energy from manures through an anaerobic fermentation process which produces a gas containing 55 to 60% methane is becoming popular. This bacterial fermentation breaks down the organic matter in manure and converts the carbon into carbon dioxide and methane with no loss of minerals. However, there is a change in the form of the minerals; for example, there is a 34% increase in ammonia nitrogen and a 47% reduction in organic nitrogen (Iannotti et al. 1979). The gas can be used as a propane replacement on the farm or as fuel for an internal combustion engine which may be coupled to an electrical generator. Innovative systems for the utilization of animal manure for the production of thermal and electrical energy have been proposed and installed in this country (Fischer, 1983; Schulte, 1983).

After processing the animal manure in an anaerobic digester, the effluent can be utilized as a feed for animals, a fertilizer for crops, or in some instances, dried and used as bedding. Ensiling the effluent with crop residues has resulted in significant animal utilization of two residues. Fertilizing soil with the effluent requires the same precautions as fertilizing with animal manures.

Combustion, gasification or pyrolysis of animal manures for energy production is only applicable when the climate is dry in order that a very fine, dry manure product can be collected.

MAJOR BREAKTHROUGHS NEEDED

Research emphasis devoted to the utilization of crop and animal residues should be multidisciplinary. The research program evaluating this technology should include biochemists, microbiologists, nutritionists, agronomists, animal scientists and engineers. Major innovative research team effort needs to be put in place for evaluation of this technology. The findings of these research teams need to be formulated into a total farming system. With the information generated, farmers can then delineate from the numerous alternatives the most efficient and effective residue management systems for specific enterprises. All organic waste can be recycled, but which is the best system for what type of recycling on a specific farm?

With computer technology being implemented on the farm, biological sensors and transducers can be coupled to electronic computers for monitoring and controling various processes. The biological parameters which will be sensed need to be defined. The sensors must accurately monitor the biological processes of converting residue into usable materials. Next, transducers which interface the defined and sensed biological process to the computer or electronic control equipment processes need development.

The efficiency of most biological conversion systems is approximately 50%. This efficiency could be improved through development of innovative and inexpensive technologies for converting residues into usable products. Since the end products of most biological fermentations are normally very dilute, novel concentrating and recovering processes that can efficiently recover the products need to be developed.

INSTITUTIONAL ARRANGEMENTS

Federal Government

The U.S. Department of Agriculture, Agricultural Research Service, Agricultural Energy Centers at Peoria, IL and Tifton, GA have supported research on utilization of crop and animal residues. Research has also been supported by the Cooperative State Research Service of the U.S.D.A. The emphasis of this work has been on conversion of residues into energy. The U.S. Department of Energy also has programs on residue conversion. These programs are at the Solar Energy Research Institute at Golden, CO., the Oak Ridge National Laboratory at Oak Ridge, TN., and Tennessee Valley Authority at Muscle Shoals, AL. Federal action agencies such as the Environmental Protection Agency require that research be accomplished to solve problems of residue effects on the environment.

State Governments

The state agricultural experiment stations provide evaluation of the localized conditions of the research. Also, a broad base of technical expertise exists on many of the land grant university campuses, where most state agricultural experiment stations are located. These multidisciplinary efforts are necessary for synergism of the effort. The state experiment stations have also supported the feeding of residues to animals.

Private Universities

Some public and private universities with disciplines and expertise necessary for given problems are involved in the research.

Private Industry

Private industries have supported this research through industry associations such as the Gas Research Institute, the Institute of Gas Technology, and various associations such as Pork Producers.

Private industry is monitoring the progress of chemical conversion but, due to proprietary concerns, exactly what action is being taken is difficult to determine.

LIKELY TIMING OF COMMERCIAL INTRODUCTION

Estimates vary for the time of commercial introduction of the technology. Economics dictate the timing for implementation of recycling crop residues and animal waste. Some of the aspects of the technology, such as, anaerobic digestion of manure for energy production are being incorporated by innovative farmers where economically feasible. Switzerland is an example of a country where energy costs are higher than in the United States. The Swiss installed over 20 digesters on farms in 1982. Whereas, in the United States approximately 20 digesters are in operation.

POTENTIAL PRIMARY IMPACTS

The greatest impact of recycling crop residues and animal waste is increased efficiency of agricultural production. Recycling will permit a higher productivity per acre of agricultural land available for production.

If the crop residues produced in the United States could be increased to the nutritive value of corn silage, an additional eleven million tons of beef could be produced per year (Klopfenstein, 1978). The utilization of the crop residue as a feed will be located geographically close to the production site due to the economy of transporting the residue any distance.

The output from processing animal manure can be electrical and thermal energy required to produce the animals from which the manure was collected (Fischer, 1981). Implementing a manure/energy conversion system required a moderate capital investment for a producer. The return on the investment is highly dependent upon economy of scale. Thus, this technology becomes economical on large animal production units. Farmers will adopt the technology based on economical feasibility which becomes more favorable with increasing energy cost. Also, efficiently and effectively disposing of manure can positively impact the environment surrounding a production enterprise through reduction of manure odors, the pollution potential of the manure and breeding habitat for insects such as flies and mosquitoes.

Recycling requires that increased processing knowledge be implemented on the farm thereby increasing the management responsibility of the farmer. The farmer can manage and engineers can design and construct successful crop residue or animal waste utilization systems if the biological sensors and transducers developed. Some of the residues might be preprocessed on the farm with final processing at a local community facility. These residue processing activities could increase gainful employment opportunities in rural America.

REFERENCES

1. Dale, B. E., "A Freeze-Explosion Technique for Increasing Cellulose Hydrolysis," 3rd Annual Solar & Biomass Workshop, Southern Agricultural Energy Center. April 26-28, Tifton, GA. 1983.
2. Dunlap, C. E., "Opportunities for Chemicals for Carbohydrates," Paper - American Institute of Chemical Engineers, Diamond Jubilee Meeting, Washington, D.C. Oct. 31-Nov. 1. 1983.
3. Federal Energy Regulatory Commission, "Small Power Production and Cogeneration Facilities: Regulations Implementing Section 210 of the Public Utilities Regulatory Policy Act of 1978," Federal Register: Vol. 45, No. 38: Monday, February 25, 1980: Rules and Regulations - pp. 12214-12237. 1980.
4. Fischer, J. R., Meador, N. F., Fulhage, C. D., and Harris, F. D. "Energy Self-Sufficient Swine Production System," TRANSACTIONS of the ASAE, 24(5):1264-1268. 1981.
5. Fischer, J. R., Iannotti, E. L., Stahl, T., Garcia, A. III., and Harris, F. D. "Bioconversion of Animal Manure into Electricity and a Liquid Fuel," Biotechnology and Bioengineering Journal, 1983.
6. Fontenot, J. P. and Webb, K. E. "Health Aspects of Recycling Animal Waste by Feeding," J. Anim. Sci.

40 :1267, 1975.

7. Fontenot, J. P., Smith, L. W. and Sutton, A. L. "Alternative Utilization of Animal Wastes." J. Anim. Sci. 57(suppl.2):221. 1983.

8. Gilbertson, C. B., Van Dyne, D. L., Clanton, C. J., and White, R. K. "Estimating Quantity and Constituents in Livestock and Poultry Manure Residue as Reflected by Management Systems," TRANSACTIONS of the ASAE. 22:(3)602-616. 1979.

9. Goedeken, F. "Utilization of Methane Digester Effluent as a Nitrogen Source in Growing Ruminant Diets," M.S. thesis, University of Missouri, May 1984.

10. Hawley, M. C., Selke, S. M., and Lampart, D. T. A. "Comparison of Hydrogen Fluoride Saccharification of Lignocellulosic Materials with other Saccharification Technologies," Energy in Agriculture, 2:219-244, 1983.

11. Iannotti, E. L., Porter, J. H., Fischer, J. R. and Sievers, D. M. "Changes in Swine Manure During Anaerobic Digestion," Dev. Industr. Microb. 20:519, 1977.

12. Kenney, V. Paul, and Erichsen, Ronald L. "Conflict Between Fuel and Food: The Ethical Dimension," Energy in Agriculture, 2:285-306, Elsevier Science Publishers B. V., Amsterdam, The Netherlands, 1983.

13. Kline, G. L., "Corn Crop Residue Collection and Conversion

for Grain Drying," 3rd Annual Solar and Biomass Workshop, Tifton, GA, April 26-28, 1983.

14. Klopfenstein, F. S. "Chemical Treatment of Crop Residues," J. Anim. Sci. 46:841. 1978.

15. Larson, W. E., Holt, R. F., and Carlson, C. W. "Residues for Soil Conservation," in Crop Residue Management Systems, (Chapter 1) American Society of Agronomy Special Publication No. 31, 677 South Segoe Road, Madison, Wisconsin, 1978.

16. Liljedahl, J. B., and Smith, R. D. "Cob Harvesting and Biomass Storage Systems," 3rd Annual Solar Biomass Workshop, Tifton, GA, April 26-28, 1983.

17. McCarty, Perry L., and Baugh, Kent. "Autohydrolysis of Organic Residues to Increase Biodegradability to Methane. 3rd Annual Solar and Biomass Workshop, Tifton, GA, April 26-28, 1983.

18. Morey, R. V., and Schertz, C. E. "Collection, Processing and Combustion of Corncobs to Provide Heat for Drying Corn," 3rd Annual Solar & Biomass Workshop, Tifton, GA. April 26-28, 1983.

19. National Academy of Sciences, Washington, D.C. 1971.

20. Paterson, J. A., Klopfenstein, T. J., and Britton, R. A. "Ammonia Treatment of Corn Plant Residues: Digestibilities and Growth Rates." J. Anim. Sci. 53(6):1592-98. 1981.

21. Prior, L. A., "Lamb Availability, Crop Production and Fertilizer Requirements in the United States," EPA/5301, SW-166, U.S. Environmental Protection Agency., Cincinnati, OH. 1975.

22. Schulte, D. D., and Splinter, W. E. Semi-Annual Progress Report, University of Nebraska Energy Integrated Farm Report. October, 1983, Project DE-FC-01-80CS-40378 U.S. Dept. Energy, 1983.

23. Smith, R. H. "Reviews of the Progress of Dairy Science," Section G. General Nitrogen Metabolism and the Rumen. J. Dairy Res. 36:313, 1969.

24. Smith, L. W. "Recycling Animal Wastes as Protein Sources," Alternative Sources of Protein for Animal Production, Proc. of a Symp., pp. 146-173, National Academy of Science, Washington, D.C. 1973.

25. Steward, G. A. Salinity Problems Associated with Wastes In: Factors Involved in Land Application of Agricultural Municipal Wastes, p. 140, ARS-USDA, Beltsville, MD. 1974.

26. Sutton, A. L., Mannering, J. A., Bache, D. H., Marten, J. F., and Jones, D. D. "Utilization of Animal Waste as Fertilizer," Purdue University, Coop. Exp. Service, Miner, ID, 101. 1975.

27. Taylor, J. C., Gable, D. A., Graber, G., and Lucas, E. W. "Health criteria for Processed Wastes," Fed. Proc. 33:1945.

1974.

28. U. S. Geological Survey, OPEN-FILE REPORT 83-728. 1983.

29. Vanderholm, D. H. "Handling of Manure from Different Livestock and Management Systems," J. Anim. Sci. 48:113, 1979.

30. Van Soest, P. J., "Refractory and Inhibitory Substances," National Ecology of the Ruminant, Chapter 8, O & B Books, 1215 NW Kline Place, Corvallis, Oregon 97330. 1982.

31. Vestal, Joe. "Broiler Litter Can Help You Winter Cattle Cheaper," Progressive Farmer. January, 1984.

32. Wilkerson, S. R. "Plant Nutrient and Economic Value of Animal Manure," J. Anim. Sci. 48:121. 1979.

Background Paper No. 9

Genetic Engineering in Plants

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Summary

Biotechnology refers to the use of the living organisms or their isolated components for industrial applications. It encompasses a wide range of techniques, including plant breeding, cell culture, cell fusion, fermentation, embryo transfer, production of monoclonal antibodies and recombinant DNA manipulations.

Biotechnology is not new to plant and animal agriculture. Plant breeding, animal husbandry, and the use of feed supplements, agrichemicals and microbial seed inoculums have made major contributions to the remarkable development of American agriculture. Within the last decade, major advances have been made in the understanding of gene function and architecture, and powerful methods have been developed for identifying, isolating and modifying specific DNA segments.

While still largely unproven, the potential for applying these biotechnology methods for increasing crop productivity is enormous. Their impact on plant agriculture will likely lead to the development of improved:

- microbial inoculums
- *in vitro* plant propagation methods
- plant varieties

Gene transfer technology in particular has great potential for the genetic modification of plants by increasing genetic diversity through the very specific modification of the expression of plant genes or the introduction of defined new genes. This technology will have major impact on the seed and plant production industries as well as the chemical, food processing and pharmaceutical industries.

The commercialization of plant biotechnology will require breakthroughs in several technical areas, including increased understanding of:

- plant cell culture
- plant transformation systems
- plant gene structure and function
- identification of agronomically useful genes
- plant breeding

Increased research funding is needed in these specific areas and generally in the basic plant sciences and molecular biology to accelerate technical development. Commercialization of plant biotechnology will also be dependent on other factors including environmental regulation, university-industry relations, economic incentives and consumer acceptance. Improved plants produced by gene transfer methods should be commercially available in 7 to 10 years. The introduction of plants produced and selected using cell culture manipulatioons and certain biotechnology-derived microbial seed inoculums or products could occur within a shorter timeframe.

Plant genetic engineering methods will initially emphasize the same targets for crop improvement (increased yield, improved qualitative traits and reduced labor and production costs) as traditional breeding programs. Ultimately the technology will lead to the development of unimagined improvement in American agriculture.

Introduction

Biotechnology refers to the use of living cells or their isolated components for industrial applications. It encompasses a wide range of methods including plant breeding, cell and tissue culture, cell fusion, fermentation and embryo transfer, which have been developed from research in the fields of genetics, microbiology, physiology, reproductive biology and cell biology. Newer techniques such as production of monoclonal antibodies and recombinant DNA manipulations are also used in biotechnological research.

Biotechnology is not new to plant and animal agriculture; the application of genetic research to plant breeding and animal husbandry has been a major contributor to the remarkable development of American agriculture. During the last several decades, biotechnology has had increasing impact on agriculture as well as the related chemical and food processing industries. For example, antibiotics, amino acids and other supplements produced by fermentation technology are routinely added to feeds to stimulate animal growth and prevent disease. Microbial seed inoculums and agrichemicals (fertilizers, herbicides, pesticides, etc.) are commonly used to increase crop productivity. Immobilized cells and enzymes are being used extensively to catalyze biochemical conversions in the production of specialty chemicals and feedstocks.

Within the last decade, major advances have been made in the understanding of gene function and architecture at the molecular level. Powerful methods have been developed for identifying and isolating specific DNA segments. Methods are now available for determining the DNA nucleotide sequence of genes and also for specifically modifying these sequences to alter gene function.

Genetic engineering methods have existed for several years for manipulating genes in microorganisms such as bacteria and yeast; however, only recently have techniques been developed for genetically modifying higher eukaryotic cells. Only within the last two years have intact mice (Palmiter et al., 1982), fruit flies (Spradling and Rubin, 1982) and plants (Horsch et al., 1984) been produced which contain and express foreign genes. It is now technically possible to introduce combinations of genes across both sexual and phylogenic barriers; the potential for using these biotechnological methods for improving productivity in both plant and animal agriculture is enormous, but still unproven. Commercial applications of biotechnology will result in major impacts on both plant and animal agriculture:

Animal Agriculture

- Disease prevention and treatment. Immunological detection of livestock diseases will be substantially improved by development of monoclonal antibodies to infectious virus and bacteria. Similar diagnostic tests are already available for detection of human diseases, but will require more cost-effective production for application to animal agriculture. Other diagnostic methods based on DNA or RNA hybridization may also be important. Vaccines produced by recombinant DNA methods have received considerable attention for prevention of diseases in livestock. Currently, vaccines against several animal diseases including foot-and-mouth disease and swine dysentery are being tested in animal trials (OTA-BA-218).

- Growth promotion and nutrition. The study of hormones and peptides which regulate specific physiological processes have been investigated extensively. The identification of classes of animal growth hormones that stimulate feed efficiency and lactation may lead to increased growth and/or milk production (Peel, et al., 1981). Efforts to commercialize such growth hormones are currently underway by several U.S. companies. The production of feed additives (antibiotics, amino acids, etc.) by fermentation technology using biotechnology-derived microbes will also have substantial impact on animal nutrition.

- Genetic modification. The feasibility of using biotechnological methods to directly modify animals has already been demonstrated in laboratory mice (Palmiter et al., 1982). These procedures, which involve the injection of foreign genes directly into fertilized eggs with small microcapillary needles followed by transplantation of the injected eggs into a surrogate female can, in principle, be applied to animal breeding programs. The methods for embryo transfer in cattle are well established and are currently used in animal husbandry; the application of such gene transfer methods to animal agriculture for increasing disease resistance and growth will require a more detailed understanding of eukaryotic gene structure and regulation. Another important area for biotechnology impact on animal husbandry is embryo sexing using monoclonal antibodies; which will prove more reliable than existing methods.

Plant Agriculture

The application of biotechnological methods such as cell culture, plant regeneration, cell fusion and recombinant DNA manipulations will also have a major impact on plant agriculture. While the impact of biotechnology on plant agriculture will lag behind its more immmediate impact on animal agriculture, its long-range effect could be even more substantial. The potential applications of biotechnology on plant agriculture include:

- Microbial inoculums. Rhizobium seed inoculums are widely used to improve nitrogen fixation by certain legumes. Extensive study of the structure and regulation of the genes involved in bacterial nitrogen fixation will likely lead to the development of more efficient inoculums. Research on other plant colonizing microbes has led to a much clearer understanding of their role in plant nutrition, growth stimulation and disease prevention and the possibility exists for their modification and use as seed inoculums. The use of microbially produced pesticides such as *Bacillus thuringiensis* spore preparations, Baculo-viruses and certain fungal insecticides has met with increasing acceptance and commercial success (Miller et al. 1983). The production of more potent or more broadly applicable microbial pesticides by biotechnological approaches seems likely.

- Plant propagation. Cell culture methods for regeneration of intact plants from single cells or tissue explants have been developed for several vegetable, ornamental, and tree species (Murashige, 1974; Vasil et al., 1979). These methods have been used to provide large numbers of genetically identical, disease-free plants which often exhibit superior growth and uniformity over conventional seed grown plants. Such technology holds promise for important forest species whose long sexual cycles reduce the impact of traditional breeding approaches. Somatic embryos produced in large quantities by cell culture methods can be encapsulated to create artificial seeds which may have advantages for propagation of certain crop species. In combination with other techniques, micropropagation methods may increase available genetic variability as well as reduce the timeframe for crop improvement.

- Genetic modification. Three major biotechnological approaches: cell culture selection, plant breeding (both traditional and newer approaches such as haploid production, use of embryo rescue, irradiated pollen, etc.) and genetic engineering are likely to have a major impact on the production of new plant varieties. The targets for crop improvement via biotechnology manipulations are essentially the same as those of traditional breeding approaches; however, the newer technology offers the potential to accelerate the rate of improvements and expand the diversity of plant traits beyond that possible by traditional breeding.

Major industries likely to be affected

The application of biotechnology to plant agriculture will have obvious direct impact on seed and plant production industries, including grain, vegetable, forage, and horticultural markets. Plant genetic engineering will result in generic modifications such as herbicide or disease resistance which can be incorporated into most species and marketed widely. In addition, the technology will produce very specific alterations in traits such as oil content or stress tolerance which may affect only very specialized markets or be applicable to limited agricultural areas. Improvements in qualitative traits (altered protein, amino acid composition, color, secondary product, etc.) are likely to be the first commercial products of biotechnology. These may require the development of special handling and marketing networks to ensure the proper separation and distribution of such "value added" materials.

The chemical industries which provide the agrichemicals (fertilizers, pesticides, herbicides, plant growth regulators, etc.) necessary to support modern agricultural practices will also be impacted by plant biotechnology. The engineering of plants resistant to specific herbicides or more responsive to plant growth regulators will extend to current application range and the specificity of these chemicals; this will require extensive coordination between the chemical industry, the seed production industry and the farmer. In contrast, the engineering of pest or disease resistance in plants by gene transfer technology and the biotechnological development of superior microbial inoculums may reduce the use of certain chemical fertilizers and pesticides. The genetic manipulation of plant enzymes or biochemical pathways may eventually lead to increased use of plant materials as chemical feedstocks (e.g., cellulose, oils, etc.) or of isolated plant enzymes in industrial processes.

The food processing industry will also be directly affected by biotechnology since traits such as protein content, sugar levels, etc. directly influence baking times, processing properties and nutritional quality. Genetic modifications affecting ripening and storage properties and stress resistance (salt, drought, etc.) will greatly influence both the timing of harvest and the processing of certain crops as well as the geographical distribution of processing centers. Since many commercial drugs and specialty chemicals (dyes, flavorings, etc.) are already derived from plants (OTA-BP-F-23), biotechnological modifications which result in higher production of secondary products or which facilitate the isolation of such products would impact both the pharmaceutical and specialty chemical industries. The ability to produce foreign proteins in plants at high levels would also be expected to impact the pharmaceutical industry and have less direct effects on several other major industries.

Plant Genetic Engineering: Current State of the Art

Of the various biotechnological methods that are being used in crop improvement, plant genetic engineering is the least established, but the most likely to have a major impact on crop improvement. Using gene transfer techniques, it is possible to introduce DNA from one plant into another plant, regardless of species and sexual barriers. For example, it has been possible to introduce storage protein genes from French bean into tobacco plants (Murai et al., 1983) and to introduce genes encoding photosynthetic proteins from pea into petunia plants (Broglie et al., 1984). The transformation technology is not limited just to the transfer of plant genes; DNA coding sequences from virtually any source can be introduced into plants providing they are engineered with the appropriate plant gene regulatory signals. Several bacterial genes have now been modified and shown to function in plants (Fraley et al., 1983; Herrera-Estrella et al., 1983; Bevan et al., 1983). By eliminating barriers to gene transfer, genetic engineering will greatly increase the genetic diversity available in plants. A major strength of the gene transfer technology lies in its molecular specificity. Genes are characterized at the DNA sequence level, thus permitting the introduction of defined DNA segment(s) into plants, without the introduction of other genes which may have undesirable effects. Using gene transfer methods it will be possible to introduce a single gene or several genes into plants in order to modify their properties. Once introduced into plants, specific molecular probes (DNA probes, monoclonal antibodies, etc.) can be used to rapidly monitor the inheritance of the new gene(s) by progeny plants in order to facilitate their introduction into elite lines.

The successful application of biotechnology methods, including gene transfer technology, to the genetic modification of crop plants will require integrated research efforts in several areas, including plant cell culture, plant transformation, plant gene structure and regulation, the identification of agronomically important genes and plant breeding. The current status of research in each of these important areas is discussed below.

Plant Cell Culture

Plant cells, like many mammalian and insect cells, can be cultured *in vitro* on defined medium. However, unlike animal or insect cells, plant cells are totipotent and can be regenerated into intact plants. The propensity for regeneration is both species, cultivar and tissue specific. Virtually all plants can be regenerated from tissue explants and organized callus, but regeneration from single cells or protoplasts is currently limited to a few genera. There are several applications of plant tissue culture which impact biotechnology research, including:

- cell culture selection of useful mutants for crop improvement
- production of specialty chemicals and pharmaceuticals
- recovery of disease free clones through meristem culture

- accelerate breeding and propagation of selected cultivars
- cellular recipients for gene transfer experiments

In general, most dicot plants, particularly the Solanaceae (tomato, tobacco, potato, petunia, etc.) have been found to be amenable to cell culture manipulation and plant regeneration (Vasil et al., 1979). Regeneration from single cells is also possible for other plants such as alfalfa, carrot, lettuce, and rape. Considerable efforts are being directed at developing regeneration systems for major commercially important dicots such as soybean and cotton. given the rate of progress and the intensity of research efforts in these areas, it is expected that plant regeneration from single cells will be possible with most dicots.

The ability to regenerate from cell culture allows for *in vitro* selection of desired traits (e.g., toxin resistance, herbicide resistance and stress tolerance) using methods (mutagenesis, replica plating, etc.) analogous to those used in microbial systems. These methods have been applied to plants such as tomato, potato, and tobacco and have allowed the selection of specific mutants, some of which are currently being evaluated in breeding programs (Chaleff, 1983).

Fusion of protoplasts from different plant cultivars or species using chemical agents allows for rapid interchange of nuclear genes. Although successful plant regeneration has been obtained from fusions between tomato and potato protoplasts and several other species (Shepard et al. 1983; Evans et al., 1983), the method appears to be limited to close plant relatives. Protoplast fusion may provide a powerful method for transferring organelles (chloroplasts, mitochondria, etc.); this may be particularly useful for transfer of traits such as male sterility or herbicide resistance between species.

An important observation that has been made in the last several years relates to the production of plant variants apparently induced by cell culture manipulation. While this variation has been used in a few cases to increase useful genetic diversity (Larkin and Scowcroft, 1981; Shepard et al., 1980), it must be controlled for other tissue culture applications where such variation could result in possibly deleterious mutations.

The culture of monocot plants has resisted many of the innovations which have led to successful regeneration systems in dicot plants. At this time regeneration from tissue explants or organized callus containing meristematic growth centers is usually possible for most monocots (corn, rice, etc.). Regeneration from single cells is, however, limited to asparagus, sugarcane, daylily and a few forage grass species (Vasil et al., 1979). Selection of mutants has been possible from organized callus tissue of corn and sugarcane and a few other monocots; however, the inability to regenerate from single cells has limited genetic selection and gene transfer approaches.

Plant Transformation Systems

Transformation of plants requires three essential components:

- Recipient -- Cell or tissue competent for DNA uptake and expression and regenerable into intact plants.

- DNA Vector -- DNA molecule which can integrate or replicate into recipient cell and which contains marker genes for identification of transformants.

- Delivery Method -- Procedure for efficiently introducing DNA vector into recipient cell or tissue.

Individual plant cells, especially protoplasts (cells treated with cell wall degrading enzymes to remove the cell wall) are the most desirable recipients for gene transfer experiments since they are available in large numbers and lack the mechanical wall barrier to DNA uptake. Genetic modification at the cellular level allows for more efficient selection and identification of transformants as well as increasing the likelihood for obtaining clonal regenerated plants.

The DNA vector which has been used in most of the successful plant transformation experiments to date is the Ti plasmid. This large plasmid is present in the soil bacterium, *Agrobacterium tumefaciens*, and has been shown to be the causative agent of crown gall disease, which infects most dicot plants (Kahl and Schell, 1982). During infection of wounded plant tissue by *A. tumefaciens* cells, a portion of the Ti plasmid, termed T-DNA is transferred into plant cells and becomes stably incorporated into plant chromosomal DNA. Recombinant DNA manipulations have allowed the construction of Ti plasmid derivatives which lack the genes responsible for crown gall disease symptoms, but allow for the transfer of the T-DNA and other passenger genes into plant cells (Chilton, 1983; Schell and Van Montagu, 1983). These modified plant vectors have been used to produce transformed plants (tobacco, petunia, tomato) which contain and express foreign genes (Horsch et al., 1984). The *A. tumefaciens* transformation system should be extendable to most commercial dicot species and with modification it may also function for monocot plants. Other potential plant vectors, such as DNA plant viruses (Buck and Coutts, 1983) and various transposable elements (Federoff et al., 1983) are being investigated. The most successful DNA delivery systems for plant cells are those which use or mimic the natural DNA transfer system of *A. tumefaciens* bacteria. Incubation of isolated plant protoplasts (Wullems et al., 1981; Fraley et al., 1983) or leaf segments (Horsch et al., 1985) with *Agrobacterium* results in a high transformation frequency. This procedure, termed cocultivation, has been used successfully with several plant species, including tobacco, petunia, potato, lettuce, rape, alfalfa, and tomato. Other DNA delivery methods, which have been successfully applied to other eukaroytic cell systems (e.g. polycation or poly alcohol treatment, calcium-phosphate precipitation, microinjection, electroporation liposome-mediated delivery) are just beginning to be successfully applied to plant cell systems. The availability of improved vectors and selectable markers should accelerate development of alternative DNA delivery methods.

Plant Gene Structure and Function

A thorough knowledge of plant gene structure is essential for successful gene transfer experiments. Both gene isolation and *in vitro* modification of gene structure and function require detailed information on DNA nucleotide sequence. In addition, obtaining proper functioning of transferred genes in new cellular and chromosomal environments requires a thorough understanding of gene regulatory processes.

Research over the last several years has revealed major differences between the structure of eukaryotic and prokaryotic genes. Many eukaryotic genes exist as multiple copies in large multigene families (5-50 genes); the various members may display differential patterns of gene expression. Unlike prokaryotic genes, the coding sequence of eukaryotic genes is not necessarily colinear and may be interrupted by noncoding regions called introns (these are eventually removed by RNA processing to form a colinear mRNA). Eukaryotic mRNA's contain long stretches of polyadenylate residues at their 3' end to facilitate RNA transport and stability; the signal for polyadenylation is contained in the DNA sequence adjacent to the 3' end of the gene. While polycistronic mRNAs (several genes under control of a single promoter) are often used to regulate related genes in prokaryotes, in eukaryotic systems each gene is controlled independently. In general, all the DNA components necessary for the temporal and tissue specific expression of eukaryotic genes is contained in the promoter region immediately 5' to the coding sequence; however, other types of regulation resulting from chromosome position effects or from DNA "enhancer" sequences are also possible.

Several studies on plant gene structure have been performed on genes encoding abundantly synthesized plant proteins such as storage proteins (Fischer and Goldberg, 1982; Schuler et al., 1982) and photosynthetic proteins (Berry-Lowe et al., 1982; Bedbrook et al., 1980; Broglie et al., 1983). In general, plant genes have the same structure and contain the same DNA regulatory sequences characteristic of other eukaryotic genes. Very little is currently known regarding the specific regulation (light induction, tissue-specific expression, etc.) of plant genes; however, the existence of gene transfer systems will allow for modification and functional evaluation of these parameters.

A key application of gene transfer technology will be to modify (increase level or alter plant tissue specificity) the expression of existing plant genes or to alter protein structure and composition. Obtaining properly regulated expression of nonplant genes in plants requires engineering plant DNA regulatory signals adjacent to the coding sequence of foreign genes. This requires thorough understanding of plant gene structure and regulation.

Selectable marker genes for plant transformation have been constructed by joining plant gene regulatory signals to bacterial genes which encode enzymes which confer antibiotic resistance; when introduced into plant cells using Ti plasmid vectors, these "chimeric genes" now confer antibiotic resistance on transformed plant cells (Fraley et al., 1983; Herrera-Estrella et al., 1983; Bevan et al., 1983).

Identification of Agronomically Important Genes

Most plant genes that have been extensively studied at the molecular level encode storage proteins or photosynthetic proteins. While these genes are of clear agronomic significance, other important genes specifying traits such as disease and pest resistance, increased yield, stress tolerance, uniform ripening, herbicide tolerance, etc., have not been extensively studied.

Efficacious use of current gene transfer technology requires a precise knowledge of gene structure and function. The reduction of a useful agronomic trait to a specific gene(s) requires both a biochemical understanding of the physiological phenomenon and the development of a suitable strategy for gene isolation. The development of new biochemical techniques for protein analysis such as micro-sequencing, monoclonal antibodies and HPLC separation methods will contribute substantially to plant gene isolation capability. In addition, the use of genetic mutants which overproduce specific gene products or which can serve as recipients for complementation experiments will be of increasing value (Chaleff et al., 1983). DNA hybridization techniques have also permitted isolation of plant genes based on their structural similarity to mammalian, bacterial, or fungal genes (Nagoa et al., 1981). Finally, the ability to introduce well characterized nonplant genes, which can result in new metabolic or catabolic activities or alter flux through existing pathways, represents a powerful approach to crop improvement. The proposed transfer of herbicide resistance genes from microbes to plants is only one example of this strategy (Comai et al., 1983).

Plant Breeding

There has been considerable confusion over the projected impact of plant genetic engineering on traditional plant breeding. Plant breeding has, and will continue to have, a major role in American agriculture. To a great extent, the success of plant breeding has depended on the ability of plant breeders to recognize and incorporate the results of basic research in plant sciences (e.g. physiology, pathology, genetics, entomology, etc.) into their programs. Biotechnology will contribute to plant breeding by both increasing available genetic diversity and by providing rapid methods for monitoring the transmission of specific genes.

Traits introduced into plants using gene transfer methods will require the same extensive agronomic evaluation as traits introduced by genetic crosses. In addition, plant breeding will be necessary to move transferred genes introduced into laboratory plants into appropriate commercial cultures and to eliminate possible deleterious mutations induced by cell culture manipulations. Plant breeders will also contribute substantially to the design of genetic engineering strategies by focusing research efforts on specific agronomically important traits and by defining new sources of these traits.

Major Breakthroughs Needed for Commercialization of Plant Biotechnology

Major Technical Obstacles

Plant genetic engineering is a new and developing field which remains confronted with several technical obstacles before its commercial applications can be realized. These obstacles are discussed below:

- Plant cell culture. Development of defined, reproducible protocols for the culture and regeneration of commercial dicot and monocot crop species is the major technical hurdle. Other key problems include the modification of plant cell culture to 1) permit more efficient isolation of genetic mutants and/or transformants, 2) allow for effective large scale culture of plant cells for production of specialty chemicals and pharmaceuticals and, 3) control cell culture induced genetic variation.

- Plant transformation systems. The major obstacle is the development of new vector systems (or the modification of existing Ti plasmid vectors) for transformation of monocot plants. Further technical development of plant vectors to allow for "shotgun cloning," cDNA expression and high copy number autonomous replication, etc., will be essential. Also of particular importance is the development of vectors for transformation of organellar (e.g. chloroplast, mitochondrion) genomes and for use with other possible transformation recipients such as pollen, eggs, meristems, etc.

- Plant gene structure and function. Generally a much more detailed understanding of plant genome structure and organization will be essential to the production of genetically modified plants. Gene sequences necessary for developmental and physiological control of gene regulation must be analyzed and understood at the molecular level. Strong emphasis must also be placed on analysis of both RNA and protein production, processing, and modification in plants.

- Identification of agronomically useful genes. The identification of useful genes requires a multidisciplinary analysis. Research programs in plant cell culture, genetics, molecular biology, and especially plant biochemistry must be expanded and coordinated to address complex physiological traits such as yield, stress response, disease and pest resistance, etc. It is essential that basic research efforts in agronomy, plant science, weed science, horticulture, and breeding be supported and interfaced with molecular and biochemical research efforts. The development of novel molecular (e.g. shotgun cloning) and genetic (e.g. transpositional mutagenesis) approaches for plant gene isolation would greatly facilitate the identification of agronomically important genes. Greater understanding of plant biochemical pathways, including their regulation and overall physiological significance, would serve to focus genetic engineering targets.

- Plant Breeding. For literally thousands of years plant breeders have effectively manipulated multigene traits without knowledge of their biochemical function. In contrast, the strength of plant genetic engineering lies in its ability to make specific predictable changes in gene structure and expression. In order to fully realize the potential of genetic engineering in plant agirculture, plant breeders and molecular biologists need to recognize and understand the strength and limitations of their respective technical approaches. It will be essential that this need be addressed by university curricula and supported by Federal granting agencies.

A key obstacle to the commercialization of genetically modified plants will be the time required for transfer of plants from the laboratory to the field for evaluation. This process can be shortened in certain crop species by using newer breeding tools (embryo rescue, haploid production, isozyme mapping, etc.). Another obstacle to comercialization involves the proprietary protection of valuable new traits introduced into plants by gene transfer technology. This concern is minimized for crop plants in which use of existing hybridization systems discourages storing seed for subsequent planting. The development of hybridization systems for other plants would facilitate general application of gene transfer technology to commercial crop species.

Other Considerations

In addition to technical problems, other factors are likely to affect the commercialization of plant biotechnology: These include:

- Research funding -- Research funding in the basic plant sciences historically has not received the priority assigned to research in health related areas. As a result, there are large gaps in the basic understanding of plant physiological and biochemical processes which will initially limit the application of genetic engineering techniques to crop improvement. This funding deficiency has been recognized in U.S. Government reports (e.g. OTA-BA-218; OTA-HR-132) and attempts are being made to correct this problem. It is important, however, that additional funding not be targeted exclusively to newer research areas in plant biotechnology, since long range efforts on identification of useful plant genes will require substantial input from the basic plant sciences.

- Technical Training -- As a result of both inadequate funding and increased industrial involvement in plant biotechnology, many highly trained scientists have left universities for industrial positions. This trend could have a serious effect on the quality of research and the training of young investigators in plant related areas. However, this effect may be partially compensated for by the entry of new investigators into the field from non-plant research areas.

- Environmental Regulation -- A major factor in the commercialization of plants derived from biotechnological manipulations will be the rate at which transferred genes can be introduced into commercial cultivars and the resulting plants evaluated under field conditions. The deliberate introduction of genetically manipulated organisms (microbes, plants, etc.) into the environment for testing is currently a topic of debate for health, safety, and environmental reasons. It is essential that existing Federal agencies (NIH

Recombinant DNA Advisory Committee, EPA and USDA) regulate release and testing under existing statutes to ensure environmental safety while allowing the United States to maintain its position as a worldwide agronomic leader. Unnecessary controls may limit the testing of biotechnologically derived plants although the introduction of specific defined DNA segments into plants using gene transfer techniques probably represents no greater cause for concern than does traditional breeding which may introduce thousands of unknown genes.

- Marketing and Distribution -- The initial plant products derived via biotechnology will likely exhibit improved qualitative or "value added" traits (herbicide resistance, altered protein levels, increased production of secondary products, etc.). Seeds or plants exhibiting these traits will be sold for a premium over conventional materials, which will require effective marketing strategies as well as special handling systems in order to separate premium materials and to ensure their proper storage and distribution.

Institutional Arrangements

Traditionally, research in basic plant sciences has been performed at State agricultural experiment stations or at land grant universities. Funding for research in the plant sciences has come primarily from government sources (NSF and USDA). In contrast, research in molecular biology and the development of gene transfer technology has developed predominantly at public and private universities in health care related areas. This research has been funded by government (NSF and NIH) and by private and industrial grants. The dichotomy that exists between the sources of funding and the location of institutional centers for plant science and molecular biology research has inhibited the emergence of strong academic research centers in plant biotechnology. In part this void has been bridged by the development of plant biotechnology research programs at major U.S. companies and bioengineering firms. However, unless university research in basic plant sciences can be effectively integrated with molecular biology technology, both the long range quality of plant-related research and the training of new investigators will be compromised.

Genetic engineering research, when successful, creates potential property rights (e.g. patents and trade secrets); the owner of such rights must decide how to best use them. In the past, the typical action of most academic researchers was rapid publication to ensure recognition as the initial discoverer of a valuable substance or process. The typical action of most corporations was to preserve trade secrecy while research and market potentials were assessed. However, these patterns are being actively reconsidered and revised by both groups. Universities are now commonly delaying publications so that patent applications can be drafted and filed; professors are recognizing that patent rights, exclusive consulting agreements, and the willingness and ability to preserve certain information in confidence can in some cases encourage the progression of an idea from a laboratory result to a commercially beneficial product. Corporations are recognizing that allowing research results to be published can lead to several advantages (greater reciprocal information flow into the corporation, increased ability to recruit high quality scientists, etc.) which must be carefully balanced against the value of trade secrets. The goal of both groups should be a carefully developed, well balanced, flexible approach toward publications, consulting agreements, collaborative research projects, and participation at scientific meetings. For a general discussion of intellectual property rights and university-industrial interactions see (Kelly et al., 1984; Division of Agriculture, Progress Report II).

The United States currently has a competitive advantage in the commercialization of plant biotechnology, both as a result of existing academic research and training programs and due to the prevailing entrepreneurial attitude of American business. Japan, the United Kingdom, Germany, and several other European countries are moving quickly to narrow this gap by increasing funding priorities and government sponsorship of biotechnology ventures. Maintaining the U.S.' competitive lead will depend on increased research support and ultimately on successful commercial development; the latter being strongly influenced by environmental regulations, economic incentives, and consumer acceptance.

Likely Timing of Commercial Introduction

Much of the biotechnological research underway in plant agriculture is highly experimental in nature. Recent scientific breakthroughs have resulted in much faster progress than was anticipated only a few years ago. However, it is important to recognize that currently there have only been two applications for field testing approval of an engineered plant (Federal Register April 15, 1983) and a soil microbe (Federal Register, June 1, 1983).

In standard plant breeding programs, depending on the particular crop species, considerable time (3 to 7 years) is involved in the introduction and testing of new traits and establishing elite lines. Additional time is required for seed increase prior to commercial release. While certain biotechnological methods may accelerate this time frame, it is difficult to foresee biotechnology derived plants produced by gene transfer technology becoming commercially available for 7 to 10 years. This time frame would seem appropriate for vegetable, forage, and horticultural species which are presently amenable to cell culture and gene transfer manipulations. Plant species which are not easily manipulated will require longer periods for commercialization.

Plants produced via cell culture selection methods may be commercialized on a somewhat shorter timescale. In some species (tomato, alfalfa, etc.) such "biotechnology derived" plants are currently undergoing field evaluation and could be released in 3 to 4 years. Commercialization of microbial seed inoculums produced by biotechnology methods may also occur somewhat more rapidly, particularly if such products are introduced into established markets (e.g. insect control, nitrogen fixation). More novel applications (weed control, plant growth enhancement, etc.) will require more extensive research and evaluation; their likely commercialization will occur in a time frame comparable to genetically engineered plants.

Potential Primary Impacts of Plant Genetic Engineering

Traditional breeding approaches to crop improvement emphasize three major targets:

- increased yield (increased biomass, disease and pest resistance, stress tolerance, etc.)
- improved qualitative traits (oil content, color, storage properties, nutritional value, etc.)

- reduced labor and production cost (herbicide tolerance, male sterility for seed production, harvestability, etc.)

These same general targets will be approached using biotechnological manipulations; however, the result will be more extreme variation in plant traits than possible by conventional techniques. Traits, such as resistance to herbicides and certain insect or viral pests and tolerance to various stresses, are currently being investigated and appear to be manipulable using gene transfer technology. Other "value added" properties such as protein and oil content, amino acid and fatty acid composition and pigmentation are also amenable to molecular modification. Complex traits such as yield, disease resistance, and nitrogen fixation are only poorly understood and will require substantial research effort before they can be predictably altered.

While there have been no commercial successes yet for genetically engineered plants or plant microbes, the applicability of these methods has been successfully demonstrated in the research laboratory. Because of the rapid progress in knowledge and technology, molecular biology will provide new tools for solving traditional agricultural problems. It will ultimately lead to the development of previously unimagined improvements in American agriculture.

References

1. Bedbrook, J., Smith, S. and Ellis, R., "Molecular Cloning and Sequencing of cDNA Encoding the Precursor to the Small Sub-unit of Chloroplast Ribulose-1,5-Bisphosphate Carboxylase," Nature 287:692-697, 1980.

2. Berry-Lowe, S., McKnight, T., Shah, D., et al., "The Nucleotide Sequence, Expression and Evolution of One Member of a Multi-gene Family Encoding the Small Subunit of Ribulose-1,5'-Bisphosphate Carboxylase in Soybean," J. Mol. Appl. Genet 1:483-498, 1982.

3. Bevan, M., O'Flavell, R., and Chilton, M-D., "A Chimeric Antibiotic Resistance Gene as a Selectable Marker for Plant Cell Transformation," Nature 304:184-187, 1983.

4. Broglie, R., Coruzzi, G., Lamppa, G., et al., "Structural Analysis of Nuclear Genes Coding for the Precursor to the Small Subunit of Wheat Ribulose-1,5-Bisphosphate Carboxylase" Biotechnology 1:5561, 1983.

5. Broglie, R., Coruzzi, G., Fraley, R., et al., "Light-Regulated Expression of a Pea Ribulose-1,5-Bisphosphate Carboxylase Small Subnunit Gene in Transformed Plant Cells," Science 224:838-843, 1984.

6. Buck, K., and Coutts, R., "The Potential of Geminiviruses in the Construction of Plant Host Vector Systems," Plant Molecular Biology 2:351-357, 1983.

7. Chaleff, R., "Isolation of Agronomically Useful Mutants from Plant Cell Cultures," Science 219:676-682, 1983.

8. Chilton, M-D., "A Vector for Introducing New Genes into Plants" Scientific American 51-59, 1983.

9. Comai, L., Sen, L., and Stalker, D., "An Altered aroA Gene Product Confers Resistance to the Herbicide Glyphosate," Science 221:370-372, 1983.

10. Division of Agriculture Committee on Biotechnology, National Association of State Universities and Land-Grant Colleges, "Emerging Biotechnologies in Agriculture: Issues and Policies," Progress Report II, November 1983.

11. Evans, D., "Agricultural Applications of Plant Protoplast Fusion," Biotechnology, May 1983, 253-261, 1983.

12. Fedoroff, N., Wessler, S., and Shure, M., "Isolation of the Transposable Maize Controlling Elements Ac and Ds" Cell 35:235-242, 1983.

13. Fischer, R., and Goldberg, R., "Structure and Flanking Regions of Soybean Seed Storage Protein Genes," Cell 29:651-660, 1982.

14. Fraley, R., Rogers, S., Horsch, R., et al., "Expression of Bacterial Genes in Plant Cells," Proc. Natl. Acad. Sci. USA 80:4803-4807, 1983.

15. Herrera-Estrella, L., De Block, M., Messens, E., et al., "Chimeric Genes as Dominant Selectable Markers in Plant Cells," *The EMBO Journal* 2:987-995, 1983.

16. Horsch, R., Fraley, R., Rogers, S., et al., "Inheritance of Functional Foreign Genes in Plants," *Science* 223:496-498, 1984.

17. Horsch, R., Fry, J., Hoffmann, N., et al., "A Simple and General Method for Transferring Genes into Plants," *Science* 227:1229-1231, 1985.

18. Kahl, G., and Schell, J., Chapters 6-21 in *Molecular Biology of Plant Tumors* (New York: Academic Press, 1982).

19. Kelly, P., Jaworski, E., Williams, J., "Agreements Covering Exchange of Biological Materials," Proceeding of American Association for Advancement of Science Annual Meeting, 1984.

20. Larkin, P., and Scowcroft, W., "Somaclonal Variation," A Novel Source of Variability from Cell Cultures for Plant Improvement," *Theo. Appl. Genet.* 60:197-214, 1981.

21. Miller, L., Lingg, A., and Bulla, L., "Bacterial, Viral, and Fungal Insecticides," *Science* 219:715-721, 1983.

22. Murai, N., Sutton, D., Murray, M., et al., "Phaseolin Gene from Bean is Expressed after Transfer to Sunflower via Tumor-Inducing Plasmid Vectors," *Science* 222:475-482, 1983.

23. Murashige, T., "Plant Propagation Through Tissue Cultures," *Ann. Rev. Plant Physiol.* 25:135-166, 1974.

24. Nagao, R., Shah, D., Eckenrode, V., et al., "Multigene Family of Actin-Related Sequences Isolated from a Soybean Genomic Library," *DNA* 2:1-9, 1981.

25. Palmiter, R., Chen, H., and Brinster, R., "Differential Regulation of Metallothionein-Thymidine Kinase Fusion Genes in Transgenic Mice and Their Offspring," *Cell* 29:701-710, 1982.

26. Pell, C., Bauman, D., Gorewit, R., et al., "Effect of Exogenous Growth Hormone on Lactational Performance in High Yielding Dairy Cows," *J. Nutr.* 111:1661-1671, 1981.

27. Schell, J., and Van Montagu, M., "The Ti Plasmids as Natural and as Practical Gene Vectors for Plants," *Biotechnology*, April, 1983.

28. Schuler, M., Ladin, B., Pollaro, J., et al., "Structural Sequences and Conserved in the Genes Coding for the α, α' and β-subunits of the Soybean 7S Seed Storage Protein," *Nucl. Acids. Res.* 10:8245-8261, 1982.

29. Shepard, J., Bidney, D., and Shahin, E., "Potato Protoplasts in Crop Improvement," *Science* 208:17-24 (1980).

30. Shepard, J., Bidney, D., Barsby, T., et al., "Genetic Transfer in Plants Through Interspecific Protoplast Fusion," Science 219:683-688, 1983.

31. Spradling, A., and Rubin, G., "Transposition of Cloned P Elements into Drosophila Germ Line Chromosomes," Science 218:341-347, 1982.

32. Vasil, I., Ahukja, M., and Vasil, V., "Plant Tissue Cultures in Genetics and Plant Breeding," in Advance in Genetics, vol. 20, pp. 127-215 (New York: Academic Press, 1979).

33. Wullems, G., Molendijk, L., Ooms, G., et al., "Differential Expression of Crown Gall Tumor Markers in Transformants Obtained after *In Vitro Agrobacterium tumefaciens*-induced Transformatin of Cell Wall Regenerating Protoplasts Derived from *Nicotiana tabacum*," Proc. Natl Acad Sci USA 78:4344-4348, 1981.

34. U.S. Department of Health and Human Services, Federal Register, p. 16459, April 15, 1983.

35. U.S. Department of Health and Human Services, Federal Register, p. 24549, June 1, 1983.

36. U.S. Congress, Office of Technology Assessment, "Impacts of Applied Genetics: Micro Organisms, Plants and Animals," OTA-HR-132, Washington, DC, April 1981.

37. U.S. Congress, Office of Technology Assessment, "Plants: The Potential for Extracting Protein, Medicines, and Other Useful Chemicals," OTA-BP-F-23, Washington, DC, Sept. 1983.

38. U.S. Congress, Office of Technology Assessment, "Commercial Biotechnology: An International Analysis," OTA-BA-218, Washington, DC, Jan. 1984.

Background Paper No. 10

Enhancement of Photosynthetic Efficiency

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ENHANCEMENT OF PHOTOSYNTHETIC EFFICIENCY

Photosynthesis is the fundamental basis for plant growth. In that process, energy from sunlight is absorbed by chlorophyll-containing tissues of the plant and used to assimilate carbon dioxide into organic molecules. The photochemical reactions in the process are intrinsically very efficient. However, several factors inhibit photosynthetic efficiency in plants: (1) certain mechanisms of photosynthesis itself, (2) the efficiency of water and nutrient use, and (3) environmental stress. Research is ongoing in each of these areas.

Plants vary in their efficiency of photosynthesis. Higher plants have an enzyme (RuBP carboxylase) that causes oxygen to react in a side reaction during photosynthesis, diverting energy that would otherwise be used to fixate carbon dioxide. This oxygenase reaction, which appears to result from a metabolic defect in plants, is encouraged by the high-oxygen, low-carbon dioxide concentration of normal air. Artificially increasing the content of carbon dioxide in the air partially suppresses this mechanism and generally results in increased crop yields. This suggests that improvements in the mechanism of photosynthesis could result in increased yields, all else being equal.

Some plants have developed a biological and morphological modification that reduces the impact of the oxygenase reaction. As a result, they waste less energy during photosynthesis. Known as C_4 plants, they include corn, sorghum, sugarcane, and millet. Plants that cannot suppress the oxygenase reaction are called C_3 plants. They include wheat, soybeans, cotton, and rice.

C_4 plants have an advantage over C_3 plants when leaf temperatures are high and a disadvantage when they are low. Moreover, C_4 plants use nitrogen and water more efficiently in photosynthesis. Thus water use efficiency could be increased in warm, arid regions if more C_4 plants could be used.

A long-term prospect for improving photosynthetic efficiency lies in research to understand the basis for the oxygenase reaction and efforts to inhibit the reaction chemically or to modify the enzyme by using recombinant DNA technology. Success will depend on many breakthroughs in understanding the chemistry and molecular biology of chloroplasts and in manipulating chloroplast genes.

Molecular biology has already yielded the ability to modify the sequence of amino acids in RuBP carboxylase to produce modified versions of the protein. This provides experimental tools of unimagined power for investigating the mechanisms of enzyme-catalyzed reactions.

Other research is being directed at improving the efficiency of use of water and nutrients through better management techniques using microcomputer-based plant growth models and new instrumentation to monitor crop performance. Improved weather forecasting will also be important. Breeding plants for efficient water and nutrient use and for stress resistance is possible and has already had some impact. These technologies have the greatest immediate prospect for improving the efficiency of photosynthesis in the next decade, although a strong research effort is needed to realize these potentials.

Introduction

Photosynthesis is the fundamental basis for plant growth and yield. Light energy absorbed by photosynthetic pigments is transformed to chemical energy and used to form the organic constituents of plants, and the energy for all other essential plant processes is obtained from respiration of photosynthetic products. The essential role of photosynthesis can be appreciated by considering that a number of very effective herbicides are specific poisons of photosynthesis. Blocking this process alone is sufficient to quickly bring about the death of most plants.

Geological evidence indicates that photosynthesis has been occurring on Earth for about 3 billion years. About 20,000 years ago human societies began to domesticate plants and develop agriculture. This step permitted mankind to make direct use of photosynthesis for production of food, fiber and fuel, and this has played an important role in the development of civilization. Breeding of crop plants since their domestication has dramatically improved the economic yield, but the photosynthetic processes of crop plants are not inherently more efficient than noncrop plants. Increased yields have occurred primarily as a result of: a) plant breeding which has increased the fraction of photosynthesis going to production of harvestable plant organs, improved the adaptation of crop plants to the agricultural environment, and increased resistance of crop plants to diseases, and b) improved cultural practices that have increased the supply of essential nutrients and water and reduced the impact of weeds and diseases.

On a global scale, mankind has captured about 2.5 percent of the net primary productivity of the biosphere, and about 10 percent of the total land area is cultivated. Cereal grains are the major crop type, accounting for 64 percent of food production in agriculture. The average productivity of the cultivated land area is only about 5 percent of what appears to be the biological potential for productivity, and the average productivity of U.S. farms is about 20 percent of the record yields for the respective crops. This chapter will examine the prospect for increasing the efficiency of this harvest of energy by mankind through the application of new technologies.

Photosynthetic Efficiency: An Overview

Our first task is to identify points where there is scope for improvement in the chain of biological reactions linking the energy of sunlight to the productivity of fields. The first events of photosynthesis are very fast photochemical reactions that occur when a quantum of light is absorbed by an assemblage of chlorophyll molecules organized to form a reaction center. Only light of wavelengths that are absorbed by chlorophyll or associated pigments can be used in this process. About 44 percent of the total energy of sunlight can be used in photosynthesis. The remainder is wasted. The photochemical reactions are very efficient in healthy photosynthetic tissue. Nearly every quantum of absorbed light leads to a reaction. For reviews see Kok (1965), Govindjee (1982).

In higher plants two similar types of photochemical reaction centers are organized in a sequence of biochemical steps that can use the energy gained in the photochemical reactions to take electrons from water producing O_2 and uses them to produce sugars starting from CO_2. A minimum of 8 photochemical reactions (hence 8 quanta of light) are required to complete the reduction of one molecule of CO_2 and produce one O_2. Assuming no waste, the best that is possible is a quantum yield for CO_2 fixation of 1/8 = 0.125 (Table 1). Another way of expressing this efficiency frequently used in agronomic and engineering literature, is to compare the energy gained per CO_2 taken up in photosynthesis (essentially the heat per carbon unit that could be obtained from burning the plant) to the heat content of the sunlight required to provide the needed 8 quanta of light. This corresponds to 12.2 percent (Table 1). This maximum efficiency is theoretically determined by fundamental properties of the pigments used, the organization of the reactions of photosynthesis, and the composition of sunlight. There is no prospect for improving the efficiency of plant photosynthesis at this level without making fundamental changes in the mechanism of photosynthesis.

Most healthy plants are capable of very efficient use of light for photosynthesis. However, this does not occur under conditions that plants typically experience. Quantum yields which approach the maximum theoretical limit are normally obtained at very high CO_2 concentrations (typically about 5 percent). In contrast normal air usually contains about 0.03 percent Co_2 and about 21 percent O_2. Under these conditions, the quantum yield for CO_2 fixation is only about half of that in 5 percent CO_2 (Table 1). This occurs because a key enzyme in CO_2 fixation, ribulose bisphosphate carboxylase (RuBisCo) cannot completely distinguish between O_2 and CO_2. When O_2 reacts instead of CO_2 an undesirable side product is formed and energy which could have been used for fixation of Co_2 is wasted. If it were possible to improve the ability of this enzyme to distinguish between the gases it would be possible to reduce this wasteful process. A major focus of work aiming to improve photosynthetic efficiency is targeted on this enzyme (Lorimer, 1981).

Some species have developed mechanisms which permit them to ameliorate the impact of this effect of oxygen. These plants are referred to as C_4 (Osmond et al., 1982), and they are marginally more efficient than normal (C_3 plants) under typical conditions (Table 1). C_4 plants also have additional advantages under arid conditions or at high temperature. This naturally occurring variation in the biochemical mechanism of photosynthesis is the most important existing means to manipulate photosynthetic efficiency. However, it is not yet possible to change pathways without also changing species.

Two other factors are important in considering the efficiency of plant leaves under normal environmental conditions. First, the efficiency of energy conversion always falls when the intensity of light is high. This is because they have a limited capacity to fix CO_2. Consequently, the process of photosynthesis may become "light saturated." The amount of light which can be used before saturation is in part a function of the nutritional status of the plant. Plants which are well fertilized can generally use higher intensities of light, and are less likely to become "light saturated" during normal days. Second, the reactions of photosynthesis are very sensitive to damage. Exposure of leaves to excessively high or low temperature, to pollutants, to too little water, or to excessively high light intensity can damage the leaves. These two factors are primarily the result of cultural practices, and farming practice has a large impact on photosynthetic efficiency by providing adequate nutrition and eliminating stresses. There is also much interest in the possibility for increasing the "stress" tolerance of plants.

We now turn to photosynthesis on a much larger scale. Consider a whole field of densely spaced young healthy plants. Measurements of daily growth rates indicate that the most productive such fields use sunlight with an efficiency of 4 to 4.5 percent (Loomis, 1982). Some losses are expected from the leaf level to the crop level because the plants respire and because some of the light reaching the crop is not absorbed. The measurements indicate that these losses under the best conditions are only about 20 percent of net photosynthesis (the single leaf level measurements indicate an efficiency of about 6 percent, as opposed to 4 to 4.5 percent for the whole crop canopy, Table 1). This is important because it shows that increasing the scale of the system from a single leaf or even isolated parts of a leaf to millions of leaves does not necessarily make things hopelessly more complicated. Plants in the field under light limited conditions seem to be able to do just about as well as plants in the laboratory. The slightly higher efficiency of C_4 plants seems to be expressed both in the laboratory and in the field.

Under normal, as opposed to optimal, conditions the overall efficiency of growth is much lower than that attained in these "high-performance fields." The average productivity of all of the cultivated lands on a yearly basis is only about 0.2 percent on the basis of the incident sunlight (Table 1). This is only some 5 percent of the efficiency that could be attained if all of the light were absorbed by healthy leaves. In the United States the average yield for the major crops is about 20 percent of the record yields for those crops (Boyer, 1982), and it is unlikely that these record yields reflect the true photosynthetic potential. It is clear that there is great scope for increasing photosynthesis if crops could attain more of their intrinsic efficiency.

A great deal of work and thought has gone into trying to understand the factors that contribute to this much lower productivity in agricultural practice, and the reader is directed to reviews by Christy and Porter (1982), Gifford and Jenkins (1982), Loomis and Gerakis (1975), Loomis (1982), Evans (1983), and Gifford et al. (1984). Most of the losses are due to incomplete interception of light which occurs when there is not a dense canopy of leaves present. There is an interval from planting time until the plants develop sufficient leaves to form a complete ground cover, and in many instances the crop fails to ever form a complete cover because of shortages of water or nutrients to support a dense canopy. Light that is not absorbed by the leaves is wasted. In addition, some of that absorbed may be wasted because of inefficiencies in the photosynthetic process itself. This can occur if leaves of the canopy have been damaged by a stress, or if the leaves become light saturated. Obviously, when potential photosynthesis is lost then potential growth and yield is also lost.

Why do these problems occur? The reasons are complex. Economic constraints play an important role. Irrigation water, fertilizers, pesticides, and intensive cultivation practices required to provide optimal conditions for the growth of crop plants are costly, and in many cases maximum photosynthetic yield does not yield maximum economic yield. Loomis (1982) states, "Agricultural production must be viewed as demand-dependent. Demand currently is such that agricultural potentials are pressed in only a few regions." The reserve capacity of agriculture is high. Using current technologies and arable land base, the productivity could be doubled and perhaps redoubled, but the cost in energy and water to realize this potential would be high.

The population of the Earth is increasing rapidly and may double by the year 2050. Increasing the production of food to keep pace with this demand may place heavy demands upon our resources, particularly the energy supply. Given this constraint it seems appropriate to ask if it may be possible to increase agricultural production without such a substantial increase in the input of resources.

Much of the increases in agricultural production that have occurred over the century has been the result of increased use of chemical fertilizers, pesticides, irrigation water, and cultivation, but recent evidence indicates that there has also been consistent plant improvement given constant cultural practices. Evans (1983) discusses the basis for improved yields in several crop species as a result of breeding for improved yield and disease resistance. He concludes that the current rate of advance (about 0.9 percent per year) in the yield potential of crops is likely to continue for some time yet.

An important factor in the past improvement of the yield potential of crop genotypes has been the "harvest index." This is essentially the fraction of total photosynthetic production by the plant that appears in the harvestable end product (e.g., seed gains, fiber, or sucrose). For example, the wild progenitors of wheat grown in the field give a harvest index of 5 to 17 percent while for a modern cultivar of wheat the harvest index is about 51 percent (Evans, 1983). Dramatic increases in the harvest index have been demonstrated for wheat, barley, peanuts, soybeans, and other crops (Evans, 1983). It is likely that there is still room for improvement in the harvest index of crop genotypes, but the scope is obviously limited since a plant needs to use some of its photosynthesis for other organs such as leaves, roots, and stems.

Increasing photosynthesis is an obvious additional strategy. Can this be done without increased inputs of resources? As noted above, there is considerable scope for increasing the efficiency of light energy conversion in photosynthesis since about half of the energy gained in the photochemical reactions is lost in reactions that occur in the low CO_2 and high O_2 concentration of the present atmosphere. Artificially increasing the CO_2 concentration of the air partially suppresses this inhibition, and generally results in increased yields (Gifford, 1982) suggesting that any improvements to the mechanism of photosynthesis would be expressed directly in increased crop yields, all else being equal. Another route that shows some promise concerns increasing the efficiency with which resources needed for crop photosynthesis are used in agriculture. To do this we might take advantage of difference in the intrinsic efficiency of resource use of different plant types or genotypes, or improve the management of resources, building upon an improved understanding of the use of resources in photosynthesis and upon improved data gathering and processing capabilities. Finally, a very promising area concerns the possibility of increasing the tolerance of crop plants to physico-chemical stresses by traditional breeding or genetic engineering approaches.

Boyer (1982) argues that there is a large potential of increasing plant performance in unfavorable environments by breeding for adaptations to tolerate the stresses present in those environments. He argues that some of the increases in agricultural productivity that have occurred in the past have been the result of pragmatic selection for better adapted genotypes. Current studies of the physiological, biochemical, and genetic mechanisms used by plants to cope with stress may permit new approaches to agriculture in habitats where stresses limit plant growth and productivity. Mechanisms that result in more efficient use of nutrients or increase the tolerance of plants to environmental stresses are very important in natural ecosystems, but have not been widely used in agriculture.

These avenues for improving the productivity of agricultural plants are discussed in more detail in the remainder of this chapter.

Basic Research on Photosynthetic Mechanisms

Most of the research in photosynthesis is basic research aimed at understanding how it works as a biological process. A review of the biochemical and biophysical mechanisms of photosynthesis was published recently by the OTA (Cramer, 1983), and this topic will not be dealt with in detail here. Instead, this discussion will emphasize research in the areas where some potential improvement seems possible. In the preceding section it was noted that the quantum yield for CO_2 uptake was substantially inhibited by the low CO_2 and high O_2 concentraion of the atmosphere, and that plants with differing mechanisms of CO_2 uptake, C_3 and C_4 plants differ in the extent of inhibition with C_4 plants being marginally more efficient than C_3 plants under normal summer conditions.

The Oxygen Inhibition of Photosynthesis. It is generally agreed that this is due to stimulation of photorespiration by the low CO_2 and high O_2 concentration of the atmosphere (Canvin et al., 1981). This occurs because O_2 can react in a side reaction with ribulose-1,5-bisphosphate (RuBP) the acceptor for the CO_2 fixation reaction in plants with the C_3 mechanism of photosynthesis. The proportion of RuBP reacting with O_2 or Co_2 (Fig. 1) is a function of the ratio of the concentrations of these two gases. A product of the oxygenation reaction is metabolized to 3-phosphoglyceric acid (3-PGA) by the PCO pathway, and a portion of the carbon is lost as CO_2. Energy that could otherwise be used to drive the reduction of fixed carbon in the PCR pathway is used in this alternative pathway (the PCO pathway). This results in a greater energy cost in terms of ATP and NADPH (or light quanta used to form them) for each net fixation of CO_2. There is no essential function for this reaction with oxygen, and it appears to be a defect in photosynthetic metabolism of plants that has become exposed as plants lowered the CO_2 and increased the O_2 of the atmosphere over past geologic epochs.

Do all plants possess this defect? Present evidence indicates that the enzyme RuBP carboxylase from all organisms possess oxygenase activity

(Lorimer, 1981), but the enzyme from higher plants seems to be somewhat less reactive with O_2 than that from some lower organisms (Jordan and Ogren, 1982). There is little known variation among crop plants. One consolation is that the increasing concentration of CO_2 in the atmosphere will tend to reduce the impact of the oxygenase.

A long-term possibility for improvement is based upon efforts to understand the basis for the oxygenase reaction and possibly inhibit it chemically or modify the enzyme using recombinant DNA technologies to reduce this undesirable side reaction. Many new breakthroughs in understanding the chemistry, and molecular biology of this enzyme are required. Success also depends upon advances in molecular biological techniques for manipulating chloroplast genes. This must be regarded as a long-term high-risk prospect, but the potential payoff is very great.

The C_4 Pathway of Photosynthesis. These plants have developed a means of reducing the impact of the defect in the enzyme, RuBP carboxylase by a biochemical and morphological modification (for a review see Osmond et al., 1982). Plants lacking this pathway are referred to as C_3 plants, and plants possessing it are referred to as C_4 plants. C_4 plants do not really lack the oxygenase activity. Instead, they modify the local concentration of CO_2 at the site of the RuBP carboxylase reaction such that the oxygenase reaction is suppressed (Osmond et al., 1982). As a result, there is less energy wasted by photorespiration in C_4 plants. The quantum yield of C_3 and C_4 plants is plotted as a function of leaf temperatures (Fig 2). C_4 plants have an advantage when leaf temperatures are high and a disadvantage when leaf temperatures are low. The fact that the quantum yields are similar to C_3 plants indicates that the additional reactions required to concentrate CO_2 about offset the losses due to photorespiration. This differential effect of temperature on quantum yield is very significant and can affect the competitive ability of grasses that have either the C_3 or C_4 pathway (Berry and Raison, 1982). These differences are probably also important in agriculture, as C_3 crops (such as wheat) are generally considered superior to C_4 crops (such as maize) at low temperatures and vice versa (Loomis, 1982). The difference could play a role in selecting between crops, all else being equal. C_4 plants are also more efficient in their use of water and the nutrient, nitrogen in photosynthesis. This will be discussed in a following section. Recent work (Lucas and Berry, 1985) has identified a functionally similar mechanism among microalgea. It is possible that this mechanism could be transferred by techniques of genetic engineering.

Molecular Biology of Photosynthesis. In the past few years there has been a dramatic increase in interest in the molecular biology of the photosynthetic system of plants. Genes coding for major proteins in photosynthesis have been isolated and sequenced. The understanding of the organization and control of genetic information of the chloroplast genome has progressed rapidly (see Whitfield and Bottomley, 1983 for a review). This work has tremendous implications for basic research on photosynthetic mechanisms. For example work on the mechanism of the oxygenase reaction of RuBP carboxylase is aided by knowledge of the amino acid sequence of the enzyme, and by the ability to experimentally modify the sequence of the enzyme using bacteria that have been genetically manipulated to produce modified versions of the protein. This provides experimental tools of previously unimagined power for investigating the mechanism of enzyme catalyzed reactions. Other questions may be similarly aided.

Nearly all of the work in molecular biology is of a basic nature, but some practical developments will certainly occur. One example is the recent development of a genetic strain of the rape seed (Brassica napus) which is resistant to the broad spectrum herbicide, atrizine. Resistant cultivars of this crop can be treated with the herbicide to control weeds, and it is essentially the only plant which will survive in the treated fields. The advantage of this is obvious.

Breeding of these resistant genotypes was directly based upon studies of the mechanism of herbicide action in photosynthesis. Experiments showed that a specific protein of the chloroplast bound the herbicide, and when bound the herbicide blocked the essential function of this protein. It was then shown that weeds which had developed resistance to the herbicide had a modified protein that no longer bound the herbicide but was still functional. This modified protein was related to a change in the corresponding chloroplast gene. This gene was transferred from a weedy species of Brassica to the crop species by special hybridization procedures designed to transfer the chloroplast gene (Charles Arnzen, personal communication). This finding opens up a whole new area of weed management that might not have been discovered by a goal oriented applied research program.

Basic research is being conducted in many institutions, including the land-grant universities, and other public and private institutions. Funding for this research comes from Federal granting agencies including the National Science Foundation, the Department of Energy, and the USDA. Special mention should be made of the Competitive Grants program within the USDA which has had a strong effect in stimulating basic research in photosynthesis. Support from private foundations has also played an important role. Private companies have recently become more interested in photosynthesis research. Some of this interest is related to the development of agro-chemicals and more recently to efforts to inhibit or eliminate photorespiration.

At the present time most of the research on photosynthesis is being done at the biochemical and physiological levels. There is a great need for more research at the whole plant and field level. In recent years, crop scientists have tended to downplay the importance of photosynthesis to crop yield, and have emphasized instead other areas concerned more directly with maintenance and defect elimination. Many of these efforts are aimed at obtaining more photosynthesis, and more direct investigation of photosynthesis at an agricultural level seems necessary.

Resource Use Efficiency

Water. About 40 percent of the land area of the United States receives too little water to permit a dense cover of healthy plants, consequently much of the light in these arid regions strikes bare ground, or is not efficiently used by plants because of effects of drought stress on photosynthesis. Productivity of various ecosystems can be predicted fairly well by considering the amount of precipitation as the limiting factor (Leith, 1975). Gates (1983) notes that the precipitation patterns may change substantially in the next few decades with climatic changes resulting from the increases in atmospheric CO_2. This may portend increased drought in the future. To some extent, irrigation of crops eliminates water limitation, but only about 5 percent of the U.S. farmland is irrigated (Boyer, 1982). Furthermore, Boyer argues that the prospect for increasing the area under irrigation is not great since the water resources are limited, and must be shared with other users. Also, irrigation brings with it problems of salinization that have plagued civilizations for thousands of years. An alternative is to make more efficient use of the water resources already available. Transport and application of water can be improved (see Boersma, this volume), and there is some scope for increasing the efficiency of water use by plants.

Terrestrial plants use a great deal of water, only a small fraction of this is directly related to photosynthesis. Most of the water used by plants evaporates inside the leaves and escapes to the atmosphere by diffusing through pores (stomata) in the epidermis of the leaves. These pores must be open when the leaf is conducting photosynthesis to permit the cells within the leaf to obtain CO_2 from the air and nutrients from the soil. Just as mammals must permit a certain amount of water to evaporate from their lungs in order to obtain the oxygen needed for respiration, plants must lose water to get Co_2 needed for growth. Plants can control the rate of water loss through physiological mechanisms that regulate the opening and closing of the stomatal pores, but plants cannot stop water loss without also causing photosynthesis to stop. Under normal summer conditions plants may lose 300 to 900 grams of water for each gram of growth (dry weight). More water is used in hot dry conditions than in cool humid conditions, and C_4 plants are generally more efficient at using water than are C_3 plants requiring about half the water for each gram of growth as a C_3 plant under comparable conditions (Fischer and Turner, 1979).

The large difference in the water requirement for photosynthesis of plants with these different mechanisms results because C_4 plants are more efficient in removing CO_2 from the air spaces within the leaf (Berry, 1975). This difference is a direct result of the different photosynthetic metabolism of C_3 and C_4 plants, and this is an example of an important biological mechanism that could be used for increasing the efficiency of water use, and therefore, photosynthesis under water-limited conditions. Water use efficiency in warm-arid regions could be increased if more C_4 crops were used. Even among plants of the same type there may be some potential for increasing the efficiency of water use (Schulze and Hall, 1982).

Drought Stress. Plants must replace the water lost from their leaves. If the leaves become even slightly desiccated the photosynthetic reactions can be damaged (Bradford and Hsaio, 1982), and with few exceptions death quickly follows severe desiccation. As plants deplete soil water, the growth pattern usually changes. Leaf growth is reduced and root growth may be stimulated even before there is any sign of stress at a physiological level. In severe cases existing leaves may be damaged or abscise. Thus, regardless of how efficiently water is used when it is available, growth can be adversely affected when the plant runs short of water. Species differ considerably in their ability to tolerate drought and recover from it when water is supplied.

Nutrients, particularly nitrogen, are also needed for plants to be productive. All reactions of photosynthesis (and other plant processes too) are catalyzed by proteins which contain nitrogen. Experiments with plants grown at different levels of nitrogen nutrition and then analyzed for their photosynthetic capacity, (Fig. 3), show that there is a very strong correlation between photosynthetic capacity and leaf nitrogen. If nitrogen is in short supply the leaves have less protein. This does not necessarily reduce the efficiency of photosynthesis at very low intensities of light, but at higher light (such as occurs in midday) a nitrogen starved leaf may not be able to use all of the light that it receives because of its lower maximum rate of photosynthesis. This phenomenon, known as light saturation, results in reduced net efficiency because some potential photosynthesis is lost during periods of high light. In addition, leaves that receive much more light than they can use for photosynthesis can be damaged resulting in a stress called photoinhibition which degrades the efficiency of the photochemical reactions of photosynthesis (Bjorkman, 1982). These effects at the leaf level may be exacerbated by responses of the plant growth pattern. With limiting nitrogen the plants form fewer leaves and more roots. The crop canopy will develop more slowly and absorb less light, thus, the overall productivity can be reduced many fold.

Significant differences among plants in water or nitrogen required for photosynthesis may prove useful. For example, C_4 plants require less water and nitrogen than do C_3 plants to attain the same rate of photosynthesis under comparable conditions. This is especially significant at high temperature. With highly productive C_4 crops such as corn and sugar cane this is often offset by more luxuriant vegetative growth, and a lower fraction of biomass going into yield. Sorghum and millet, C_4 crops of the nutrient-poor, arid subtropics probably derive much greater relative benefit from the C_4 mechanism. Presumably, corn could be bred to attain higher efficiences of nitrogen or water use.

Management of Nutrients and Water. In order to obtain maximum photosynthetic efficiency from crop plants it is necessary to provide luxuriant nutrition and sufficient water. Very effective management techniques for achieving this have been developed from practical experience and applied research programs. Limitations on resource and economic constraints, however, impose a more difficult strategy of accepting reduced efficiency, productivity and yield to control the costs of production. In this context the overall efficiency of solar energy conversion may not be the most relevant index to use, since the availability of other resources for plant growth are limiting. This should not be taken to imply, however, that photosynthesis is not important. The harvested crop is generally proportional to the photosynthetic productivity, and resources such as nitrogen and water have a direct role in determining the amount of photosynthesis. It has been suggested that agricultural research should be striving to increase the efficiency with which limiting resources (e.g. water, or nitrogen) are used in crop production (Berry, 1975). According to this argument, the objective should be to obtain the maximum photosynthesis with a given limiting resource.

This is a complex problem. If resources are applied sparingly to avoid waste, then there is a greater chance that the crop may run short of nutrients or water during the growing season, causing a stress which could lead to a sharp reduction in yield. To further complicate things the rainfall and weather events such as hot drying winds cannot be controlled.

Like any other management problem, more and better information would be helpful. A great deal of farm management information is already being obtained from analysis of the soil, the composition of the plants and the weather. These permit tracking of the resource use by crops, but this would be much more useful with accurate projections of future needs and rainfall. It seems certain that the accuracy of long and short range weather forecasting will improve in the next decades. This might permit a more informed choice of a crop variety appropriate for the coming year. Also, at a more sophisticated level, the weather information might be used in crop simulation models to forecast crop growth and resource use permitting managers to anticipate problems before they develop and permitting alternative management strategies to be evaluated. Computer models that simulate crop growth have been developed and are being used for research purposes (for a review see Penning de Vries, 1982). Applied uses of such models should become more feasible because the models are becoming simpler and less cumbersome, and because the computational power of microcomputers is increasing dramatically. Computer techniques will certainly find application in collecting, organizing, and analyzing data useful in crop management. Increased application of such technologies should lead to steady improvement in crop management efficiency.

Stress Resistance in Crops

Environmental stress factors can play an important role in restricting plant yield especially when economic or environmental considerations limit the resources available for crop production. Management practices to mitigate stresses is an important approach to this problem. Another approach is to take advantage of biological mechanisms that permit some plants to tolerate unusual levels of stress. Many natural ecosystems (for example salt marshes and tropical savannahs) maintain high productivity in environments that would severely restrict the growth of crop plants. These native plants apparently have physiological, morphological and developmental mechanisms lacking (or underdeveloped) in crop plants that enable them to cope with higher levels of salinity or drought. There is great variation among plants in their response to stresses such as drought, salinity, or temperature extremes, and this provides a biological resource that may be used for reducing the impact of these stresses.

It seems likely that crop plants can be bred to have greater resistance to stress, and Boyer (1982) argues that a significant proportion of the genetic improvement of crops such as corn over the past decades has occurred by improved genetic adaptation to the agricultural environment. Boyer (1982) cites an example of improved progress in breeding for drought resistance in wheat by selecting for physiological traits of osmotic adjustment to cellular water deficit that have recently been identified as being important in drought tolerance. Without such knowledge and a selection regime to screen for it, it would have been more difficult to identify the drought tolerant genotypes. Success in breeding for salinity tolerance in tomato was aided by crossing the commercial cultivar with genetically compatible native species of Lycopersicon that occur in saline habitats, and breeding for salinity tolerance in wheat was aided by identification of natural variation in salinity tolerance within the existing gene pool (Epstein et al., 1980). Single cell selection for salinity tolerance in tissue culture is also a promising possible route (Wynn Jones and Gorham, 1983).

The mechanisms used by plants to cope with stress are generally complex and are not yet well understood at a physiological or genetic level. Consequently, it is often difficult to breed for stress tolerance. Boyer (1982) argues that there is a pressing need for research into the mechanisms of stress tolerance. This work should be focused on crop plants and upon plants native to stressful natural environments. The work with stress tolerant native plants is important to identify and characterize the biochemical, physiological, and genetic mechanisms that work in natural environments. The results of research with native plants can be used in crop improvement studies by helping to identify the "design principles" that could be used, and may be helpful in identifying appropriate genetic variability within the gene pools of crop species. In this regard, expanded research into the biology of desert plants would seem appropriate, since many of the environmental stresses affecting agricultural production are extreme in desert regions.

Research of this type is being conducted in only a few laboratories, but there is an expanding interest in stress physiology. Research is being funded by the Federal granting agencies, and the USDA. Researchers within the agricultural institutions are generally restricted to working with particular crop species, and much of the work has focused upon the mechanisms of stress damage rather than upon mechanisms of stress resistance. There is need for expansion of interdisciplinary work on mechanisms of adaptation. We need to know how adaptations function at the whole plant level and how this is related to the biochemical and genetic components of the adaptation. Plant improvement work is still generally at the "trial and error stage." This can work well with simple genetic traits, but attempts to engineer complex adaptive mechanism into crop genotypes will be greatly facilitated by a more analytical approach based upon research on the "design principles" that have been successful in plants subject to natural selection in stressful natural habitats.

Summary

Photosynthesis is the fundamental basis for plant growth. Energy from sunlight is used to form the organic constituents needed for plant growth and respiration. The chain of events linking light interception by pigment molecules to net primary productivity at the level of a crop in the field was analyzed to identify possible sites of improvement. The photochemical reactions are intrinsically very efficient, but only about half of the energy in sunlight can be used in these reactions. Drought, high or low temperature or photoinhibition can damage the photochemical reaction centers. Biochemical reactions linking the photochemical reactions can be very efficient, but O_2 can participate in a reaction that results in waste of energy that could have been used for CO_2 fixation. In normal air about 40 percent of the energy captured in the photoacts is lost in ractions linked to oxygen. All evidence indicates that this is a defect in metabolism. Research aimed toward understanding the chemical basis of this reaction and possible means of modifying the enzyme that does this reaction are in progress. This research could have a large benefit, but a breakthrough is required. No practical application of this research is likely in the next decade. A biological mechanism which suppresses the inhibition, known as the C_4 pathway of photosynthesis, results in improved efficiency relative to normal plants (with the C_3 pathway) at high temperature (>30°C), but the situation is reversed at lower temperature. C_4 plants appear to have an advantage in hot climates.

These studies with leaves under ideal conditions indicate a maximum efficiency of about 6 percent (based on the heat content of sunlight). Large fields of plants growing under ideal conditions can achieve 4 to 4.5 percent efficiency on the same basis. Only by increasing the biological efficiency of photosynthesis could photosynthesis in these fields be increased. In general, however, the productivity of fields is not as high as observed in these "super fields." Much of the light reaching typical fields is not absorbed, and the plants themselves may be inefficient as a result of nitrogen deficiency or stress. In order to improve photosynthetic productivity under these conditions it is necessary to improve the efficiency of photosynthesis on the basis of the limiting resource.

Both water and nitrogen are required for photosynthesis and it is possible to consider the efficiency of use of these resources in quantitative terms. These efficiencies can be improved by better management techniques using microcomputer based plant growth models, and new instrumentation to monitor crop performance. Improved weather forecasting will also be important. In addition, plants can be bred to increase the efficiency of water or nitrogen use. Breeding plants for increased tolerance to stresses such as drought, salinity, or temperature extremes is possible and has already had some impact. These technologies have the greatest immediate prospect for improving the efficiency of photosynthesis in the next decades. A strong research effort is needed to realize these potentials.

References

Berry, J.A., "Adaptation of Photosynthetic Processes to Stress," Science 188:644-650, 1975.

Berry, J.A., and O. Bjorkman, "Photosynthetic Response and Adaptation to Temperature in Higher Plants," Ann. Rev. Plant Physiol. 31:491-543, 1980.

Berry, J.A., and W.J.S. Downton, "Environmental Control of Photosynthesis," in Photosynthesis: Development, Carbon Metabolism and Plant Productivity, Govindjee (ed.) (New York: Academic Press, 1982).

Berry, J.A., and J.K. Raison, "Responses of Macrophytes to Temperature," in Physiological Plant Ecology I. The Encyclopedia of Plant Physiology Vol. 12A, O.O. Lange, P.S. Nobel, and H. Ziegler (eds.) (Berlin: Springer-Verlag, 1982).

Bjorkman, O., "Responses to Different Quantum Flux Densities," ibid, 1982.

Boyer, J.S., "Plant Productivity and Environment," Science 218:443-448, 1982.

Bradford, K.J., and H.C. Hsaio, "Physiological Responses to Moderate Water Stress," in Physiological Plant Ecology II. The Encyclopedia of Plant Physiology 12B, O.L. Lange, P.S. Nobel, C.B. Osmond, and H. Ziegler (eds.) (Berlin: Springer-Verlag, 1982).

Canvin, D.T., J.A. Berry, M.R. Badger, H. Fock, and C.B. Osmond, "Oxygen Exchange in Leaves in the Light," Plant Physiol. 66:302-307, 1980.

Christy, L.A., and C.A. Porter, "Canopy Photosynthesis and Yield in Soybean," in Photosynthesis: Development, Carbon Metabolism and Plant Productivity, Govindjee (ed.) (New York: Academic Press, 1982).

Cramer, W.A., "Enhancement of Photosynthetic Efficiency," in Emerging Technologies in Agricultural Production, Y. Lu (ed.) Office of Technology Assessment, Washington, DC, Oct. 1983.

Ehleringer, J.R., and O. Bjorkman, "Quantum Yields for CO_2 Uptake in C_3 and C_4 Plants. Dependence on Temperature, CO_2 and O_2 Concentration," Plant Physiol. 59:86-90, 1977.

Ehleringer, J.R., and R.W. Pearcy, "Variation in Quantum Yield for CO_2 Uptake Among C_3 and C_4 Plants," Plant Physiol. 73:555-559, 1983.

Epstein, E., J.D. Norlyn, D.W. Rush, R.W. Kingsbury, D.B. Kelly, G.A. Cunningham, and A.F. Wrona, "Saline Culture of Plants: A Genetic Approach," Science 210:399-404, 1980.

Evans, L.T., "Raising the Yield Potential: By Selection or Design?" in Genetic Engineering of Plants: An Agricultural Perspective, T. Kosuge, C.P. Meredith, and A. Hollanender (eds.) (New York: Plenum Press, 1983).

Farquhar, G.D., and S. von Caemmerer, "Modeling of Photosynthetic Response to Environmental Conditions," in Physiological Plant Ecology I. Encyclopedia of Plant Physiology Vol. 12B, O.L. Lange, P.S. Nobel, C.B. Osmond, and H. Ziegler (eds.) (Berlin: Springer-Verlag, 1982).

Fischer, R.A., and N.C. Turner, "Plant Productivity in Arid and Semiarid Zones," Ann. Rev. Plant Physiol. 30:277-317, 1978.

Gates, D.M., "Chapter 2. An Overview," in CO_2 and Plants," E.R. Lemon (ed.) (Washington, DC: AAAS Selected Symposium 84, Am. Assoc. for the Adv. of Science, 1983).

Gifford, R.M., "Global Photosynthesis in Relation to Our Food and Energy Needs," in Photosynthesis: Development, Carbon Metabolism and Plant Productivity, Govindjee (ed.) (New York: Academic Press, 1982).

Gifford, R.M., and C.D. Jenkins, "Prospect of Applying Knowledge of Photosynthesis to Improving Crop Production," ibid., 1982.

Gifford, R.M., J.H. Thorne, W.D. Hitz, and R.T. Giaquinta, "Crop Productivity and Photoassimilate Partitioning," Science, 1984.

Govindjee [no initial] Photosynthesis: Energy Conversion by Plants and Bacteria (New York: Academic Press, 1982).

Jordan, D.B., and W.L. Ogren, "Species Variation in the Specificity of Ribulose Bisphosphate Carboxylase/Oxygenase," Nature 291:513-515, 1981.

Kok, B., "Photosynthesis: The Path of Energy," in Plant Biochemistry, J. Bonner, and J.E. Varner (eds.) (New York: Academic Press, 1965).

Leith, H., "Modeling the Primary Productivity of the World," in Primary Productivity of the Biosphere, H. Leith, and R.H. Whittaker (eds.) (Berlin: Ecological Studies 14, Springer-Verlag, 1975).

Loomis, R.S., "Productivity of Agricultural Systems," in Physiological Plant Ecology III. Encyclopedia of Plant Physiology Vol. 12C, O.L. Lange, P.S. Nobel, C.B. Osmond, and H. Ziegler (eds.) (Berlin: Springer-Verlag, 1982).

Loomis, R.S., and P.A. Gerakis, "Productivity of Agricultural Ecosystems," in Photosynthesis and Productivity in Different Environments, J.P. Cooper (ed.) (Cambridge: Cambridge University Press, 1975).

Loomis, R.S., R. Rabbinge, and E. Ng, "Explanatory Models in Crop Physiology," Ann. Rev. Plant Physiol. 30:339-367, 1979.

Lorimer, G.H., "The Carboxylation and Oxygenation of Ribulose-1,5-Bisphosphate: The Primary Events in Photosynthesis and Photorespiration," Ann. Rev. Plant Physiol. 32:349-383, 1981.

Lucas, W.J., and J.A. Berry, Inorganic Carbon Uptake by Aquatic Photosynthetic Organisms (Rockville, MD: The American Society of Plant Physiologists, 1985).

Osmond, C.B., K.H. Winter, and H. Ziegler, "Functional Significance of Different Pathways of CO_2 Fixation in Photosynthesis," in Physiological Plant Ecology II. Encyclopedia of Plant Physiology Vol. 12B, O.L. Lange, P.S. Nobel, C.B. Osmond, and H. Ziegler (eds.) (Berlin: Springer-Verlag, 1982).

Penning de Vries, F.W.T., "Modeling of Growth and Production," ibid. 1982.

Schulze, E.D., and A.E. Hall, "Stomatal Responses, Water Loss and CO_2 Assimilation Rates of Plants in Contrasting Environments," ibid. 1982.

Whitfield, P.R., and W. Bottomley, "Organization and Structure of Chloroplast Genes," Ann. Rev. Plant Physiol. 34:279-310, 1983.

Wong, S.C., Stomatal Behavior in Relation to Photosynthesis (Canberra, Australia: Ph.D. Thesis, The Australian National University, 1979).

Wyn-Jones, R.G., and J. Groham, "Aspects of Salt and Drought Tolerance in Higher Plants," in Genetic Engineering of Plants: An Agricultural Perspective, T. Kosuge, C.P. Meredith, and A. Hollanender (eds.) (New York: Plenum Press, 1983).

Table 1.--The efficiency of the photosynthetic process at several levels of organization expressed on the basis of quantum yield, (CO_2 fixed per quantum of light absorbed) or energy yield of plant biomass relation to the heat content of the light used.

Level of organization	Quantum yield	Energy yield
Photochemistry	0.125	12.2%
Whole organisms		
Algae at 5% CO_2	0.10	10 %
C_3 plant, air	0.054	5.3%
C_4 plant, air	0.065	6.3%
Crops (maximum daily growth rate)		
C_3 plants	--	4.0%
C_4 plants	--	4.4%
Yearly productivity		
Maximum crop growth	--	2.5%
Average cultivated area	--	0.22%
Global average	--	0.12%

FIGURE 1. Schematic diagram indicating the competing reactions of CO_2 and O_2 in photosynthetic metabolism. Energy from the photoacts is used to drive both pathways (the PCR pathway and the PCO pathway), but only the PCR pathway results in net CO_2 uptake. The PCR pathway is counter productive (from Berry and Downton, 1982).

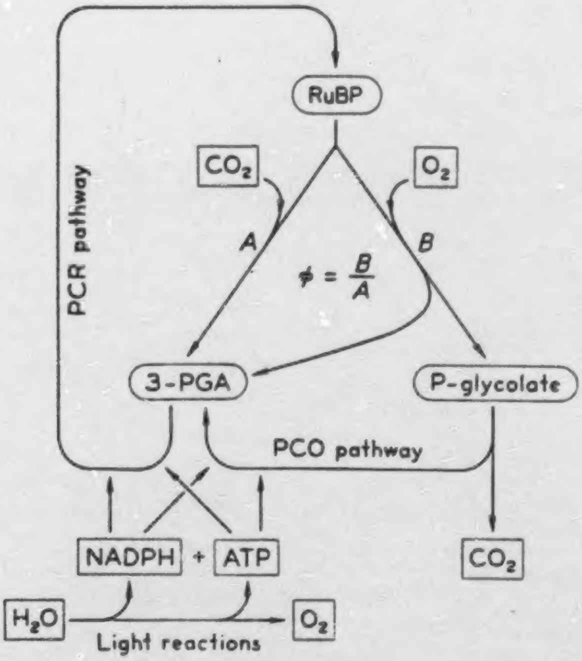


FIGURE 2. The quantum yield for net CO_2 uptake by C_3 and C_4 plants as a function of the CO_2 and O_2 concentration of the air (left panel) and temperature at normal CO_2 and O_2 concentrations (right panel). (Data from Ehleringer and Björkman, 1977.)

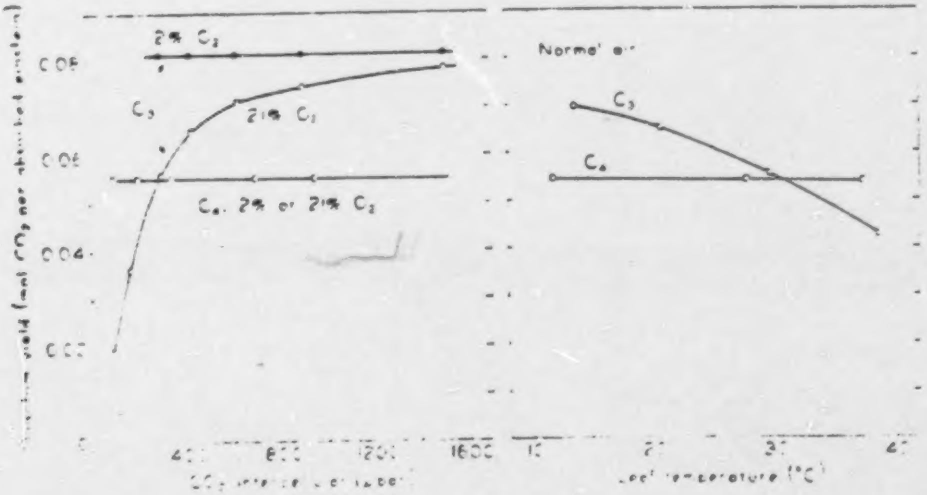
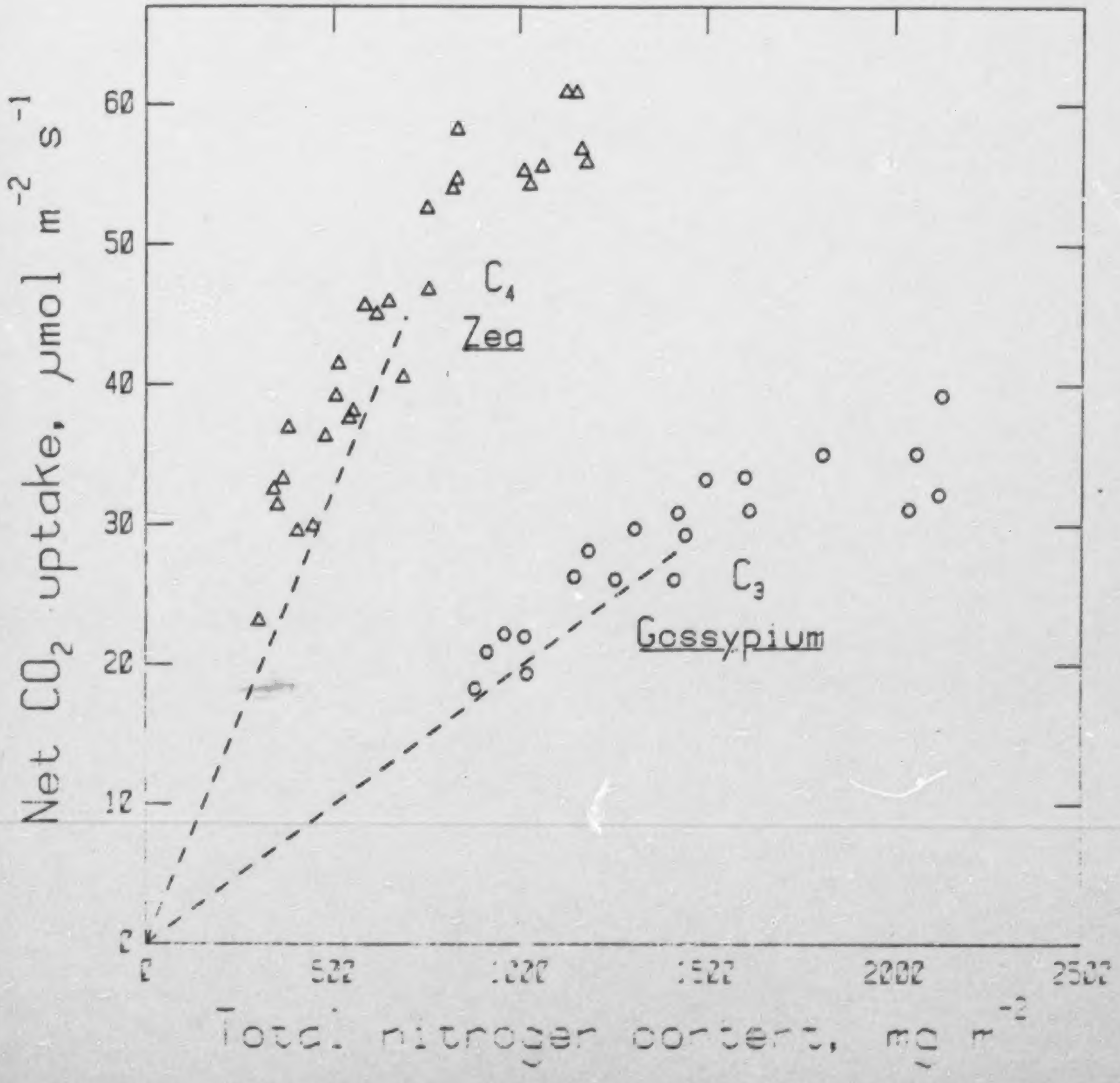


FIGURE 3. Net CO_2 uptake by intact leaves of _Zea mays_ and _Gossipium hirsutum_ at rate-saturating light and 30°C as controlled by the nitrogen content per unit leaf area. Each point is for a different leaf that developed under different nitrogen nutrition (from Wong, 1979).



Background Paper No. 11

Plant Growth Regulators

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PLANT GROWTH REGULATIORS

Summary

Plant growth regulators are natural or synthetic compounds that are applied (usually directly) to a plant to alter its life processes or structure in order to improve quality, increase yields, facilitate harvesting, or any combination of these. Used commercially since the 1920's, plant growth regulators have had a variety of impacts. One of their earliest was in rooting powders and solutions for the propagation of cuttings. Another was the impact of maleic hydrazide on the prevention of sprouting in potatoes and onions during storage.

The biggest boost to plant growth regulation came with the discovery that phenoxyacetic acids kill broadleaved plants but not grasses. This use of chemicals in herbicides has out distanced economically all other uses of plant regulators and, until recently, dwarfed their general importance.

Overall research on plant growth regulation is currently multipronged. Industrial research is particularly directed at two major U.S. crops--corn and soybeans.

An increasingly important research effort is that for antidotes to herbicides. Called protectants, or safeners, such compounds can be applied to the crop, usually to the seed, to make it resistant to an herbicide. When the herbicide is applied to the crop row, it kills only the weeds.

The U.S. Department of Agriculture has used plant growth regulators so successfully in the guayule bush that it may be theoretically possible to have a rubber industry within the boundaries of the United States. Ethephon is used to prevent coagulation of latex flow in rubber trees, eliminating the need to tap the trees daily. Plant growth regulators of the triethylamine type are used to increase the total rubber content of the guayule bush. A similar use of growth regulators is the use of paraquat on pine trees. The result is a significant increase in oleoresin content and the possibility that the naval store industry may take on new life in the Southeast United States.

The success in the sugarcane industry in the control of flowering, in the use of gibberellic acid to increase the tonnage of both cane fiber and sugar, and in the use of ripeners to enhance sugar yields allows that industry to turn to research using dessicants as harvest aids.

In the grape industry the successful use of gibberellins on grapes is stimulating studies on the control of abscission (the shredding or separating of plant organs such as fruit or leaves) and the use of ripeners to increase sugar content. Abscission agents have been used successfully on cotton, oranges, cherries, and olives, where it reduces the tenacity of the fruit sufficiently to allow easy harvest by hand-picking, mechanical harvest, or shaking. Abscission agents have also been used to thin apple blossoms, changing the yield pattern from alternating light-fruiting and heavy-fruiting years to annual, successfully bearing years.

Plant growth regulators can reduce harvesting costs by changing the shape of the whole plant or just its fruit to allow easier mechanical harvesting. Apples, grapes, and wheat are examples. Gibberellic acid is used with grapes, for instance, to lengthen the pedicel to each berry. This reduces the rotting that normally occurs because grapes grow too close together. The size and shape of both apples and grapes can be changed by cytokinins and gibberellic acid.

Regulators can also be used to speed or delay maturity of fruit. Success has already been notable with Navel oranges and with pineapple, peppers, cherries, coffee, tomatoes, and tobacco. In addition, the tremendous losses of food crops following harvest almost guarantees an increase in research to develop preharvest and post-harvest preservation through plant growth regulators.

Finally, preliminary indications with Cycocel and other chemicals suggest that overcoming environmental limitations via plant growth regulators should be a fertile field for investigation.

A substantial number of new products or new uses for existing products can be expected in the 1990's. Because of the difficulty in registering new compounds, many of the advances will be extensions of uses of existing products. Since so much of the chemistry, evaluation, and expensive toxicology has already been done on existing products, finding new uses for those products might well have a greater impact than researching new compounds.

TECHNOLOGY, PUBLIC POLICY, AND THE CHANGING STRUCTURE OF AMERICAN AGRICULTURE

PLANT GROWTH REGULATORS

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I. INTRODUCTION

A. Definition and Scope

It has been established, and is now well accepted, that normal plant growth and development is controlled by chemicals produced by the plant itself. The production of these chemicals is, in turn, under genetic control. These chemicals are called hormones. It is also apparent that synthetic growth regulating chemicals are becoming increasingly important and valuable in the commercial control of crop growth, both in agriculture and in horticulture. The general thinking is that some synthetic growth-regulating chemicals produce their effects through changing the internal levels of the naturally-occurring hormones -- thereby causing a modification of growth and development in the desired direction and to the desired extent. Other growth regulators act as hormones, or actually are duplicates of the natural hormones, and regulate genetic or biochemical systems to control plant growth. Much attention has been given in the popular press to the potential for the use of plant growth regulating chemicals in controlling crop plants as well as horticultural plants; the predictions for success range from a reluctant conservatism to unlimited enthusiasm. The truth probably lies somewhere in between these extremes.

Plant growth regulators are usually defined as organic compounds, other than nutrients, that affect the physiological processes of growth and development in plants when applied in low concentrations. For practical purposes, they can be defined as either natural or synthetic compounds that are applied directly to a target plant to alter its life processes or its structure to improve quality, increase yields, facilitate harvesting, or any combination of these. Put another way, plant growth regulating chemicals should be able to be used to modify a crop by changing the rate or pattern or both of its response to either the internal or the external factors that govern all (or any) stages of crop development from germination to vegetative growth, reproductive development, maturity, and senescence (or aging), as well as post-harvest preservation.

What is the history of plant growth regulators? Plant growth regulators are not new. The use of chemicals to induce flower formation was discovered by accident many years ago. Apparently, an accidental fire in a greenhouse in the Azores in 1893 caused the owner to think his plants had been ruined. He was surprised, indeed, when he found that most of the plants in the greenhouse subsequent to the fire burst into bloom. This observation was followed by the general use of smoke to induce flowering in several plantation crops, including pineapple. This successful use, of course, was followed by a search to find out what the active materials were, and it was found that unsaturated gases such as acetylene, ethylene, etc. were the cause of the floral induction. Plant growth regulators have been used commercially since the 1920's. One of their earliest uses was in the induction of rooting. Almost everyone is familiar with rooting powders and rooting dusts that are available both for soft- and hardwood cuttings. Early in the study of rooting of cuttings for propagation, it was found that the compound indolebutyric acid was the most effective compound for this purpose. It is interesting that in the intervening 50 or more years we have not found another compound more effective than this one, although we have found others that have considerable activity.

Plant growth regulation probably got its biggest boost in the early 1940's with the discovery -- by groups both in the United States at Boyce Thompson Institute and at ICI's experiment stations in Great Britain -- that the phenoxyacetic acids have a differential effect on broadleaf plants as opposed to narrow-leaved or grass-like plants. They found that broadleaved plants were killed by compounds such as 2,4-D and MCPA, whereas grasses were untouched. This discovery, plus the fact that World War II caused a severe reduction in the labor force, made the introduction of these early herbicides an extremely important aspect of agricultural history. The success of herbicides is probably due both to the technical discovery as well as its economic timing. In fact, the use of chemicals for herbicidal purposes has outdistanced economically all uses of chemicals for the regulation of other plant processes and dwarfed their general importance. The economic success with herbicides has been so great, because of the savings in labor costs, that the study of plant growth regulation has in fact, until very recently, been an orphan of herbicide research programs.

Another of the early and well known successes of plant growth regulation was the use of compounds such as maleic hydrazide for the prevention of sprouting of potatoes and onions during storage. For many years this was the largest use of plant growth regulating chemicals.

B. Major Lines of Research

The overall research effort in plant growth regulators is currently multi-pronged. Industrial research is aimed primarily at major crops where success will bear the considerable cost of the research and development necessary to achieve success. Emphasis is particularly directed at two of the major U.S. crops, corn and soybeans, both outstanding for the lack of success achieved to date in controlling growth and development through the use of chemicals. Antidotes (often called protectants or safeners) to herbicides is a major research effort at the present time and promises to be an increasingly important one in both the immediate and distant future.

The success by the USDA team in California in increasing the rubber content in guayule, making it theoretically possible to have a rubber industry within the boundaries of the U.S., makes this an extremely promising area for continued emphasis. The success in stimulating the flow of latex in the *Hevea* rubber industry should spur efforts to increase yields of latex *per se*. Preliminary success in inducing resistance to diseases and insect pests by the use of chemicals and in the overcoming of environmental stresses such as drought, salinity, and cold opens up an extremely fertile area for government, academic, as well as industrial research efforts -- in which increased emphasis is already becoming apparent.

With the success in the sugarcane industry in the control of flowering, the use of gibberellic acid to increase cane tonnage as well as sugar and the use of ripeners, allows that industry to turn to increasing tillering and the use of dessicants as harvest aids as their future research targets. The successful use of gibberellins on grapes is stimulating studies on the control of abscission and the use of ripeners to increase their sugar content. The tremendous losses of food crops following harvest almost guarantees an increase in the efforts to obtain post-harvest preservation through the use of chemicals applied both pre- and post-harvest.

II. CURRENT STATE OF THE ART

In the late 1950's it was found through basic studies on photoperiodism in sugarcane that cane was very sensitive to night interruption by light. As this approach could not be used commercially, the use of chemicals to limit the effect was studied in Hawaii. It was soon found that compounds such as CMU and DCMU, when applied at approximately 4 lb a.i./A, were successful in preventing 80 to 90% of the flowering of the cane varieties used at that time. Subsequent studies found increasingly more active compounds, ultimately resulting in diquat being the standard practice at the present time, where 1/8 of 1 lb a.i./A will control 100% of flowering. More recent work indicates that ethephon might be even preferable to diquat.

Abscission, the shedding or separation of plant organs such as fruit or leaves, has important impacts on crop yields and quality. The first commercial use of abscission agents was that of naphthaleneacetic acid to thin apple blossoms. Prior to these thinning procedures, there was a biennial bearing of trees in the apple industry -- a heavy fruiting year followed by a light one. By controlling the amount of fruit which was allowed to mature, it was found that yields could be evened out, and every year could be made to be a successful bearing year. Subsequently, the abscission or removal of leaves from cotton was found to be advantageous for mechanical harvesting. Studies showed that a number of compounds induced the abscission of cotton leaves. At the present time, over 80% of the American acreage is treated with either abscission agents or dessicating agents. The use of abscission agents applies also to oranges, cherries, and olives, where the tenacity of the fruit is reduced sufficiently to allow easy harvest either by hand-picking, by mechanical picking, or by shaking.

Plant growth regulators have been used to change the physiognomy of the whole plant and of the fruit. The shape of young apple trees can be changed making them the desired shape low to the ground, the apple fruit can be changed in shape by a combination of gibberellins and cytokinins, and the shape of grape bunches can be improved by treating with phenylurea-type cytokinins and/or gibberellic acid, which causes an increase in the length of the pedicel to each individual berry resulting in less rotting of fruit because of the closeness of the berries in normal development. Other changes in physiognomy of the whole plant include the shortening of cereals such as wheat by the use of chlormequat and ethephon. These cause a shortening of the vegetative straw but no change in the flowering head. The end result is not an actual increase in productivity but a significant increase in harvest recovery because wind and rain do not cause a lodging of the cereal and a consequent loss to rotting.

The effect of gibberellic acid is primarily an elongation of the main axis. This fact has been utilized in certain crops to increase the economic value of that crop, as in the case of sugarcane. The control of sex, whether male or female flowers are produced, can be achieved by the use of the proper plant growth regulator, especially in the cucurbits.

Maturity of fruit is controlled commercially in many crops by the use of the appropriate plant growth regulator. An outstanding example is the control of the maturity or the delay of the maturity of Navel oranges by gibberellic acid in the

California citrus industry. Ethephon can be used to control the maturation of pineapple, peppers, cherries, coffee, tomatoes, and tobacco.

Another outstanding and now well established use of plant growth regulators is the control of latex flow in Hevea rubber trees. Ethephon is used throughout plantations producing rubber to prevent the coagulation of latex flow. This has resulted in a significant decrease in cost of rubber production because, instead of having to tap at daily intervals, this is done much less frequently. Plant growth regulators of the triethylamine type are used to increase the total rubber content of the guayule bush, which grows in the southern United States and northern Mexican desert area. It is estimated that the increase might be sufficient to make this plant an alternate source of rubber. Another metabolic effect is that of paraquat on pine trees resulting in a significant increase of the oleoresin content. This increase appears to be enough so that the naval stores industry might take on new life in the southeast United States.

Another major partitioning effect is in the use of plant growth regulators for the ripening of sugarcane. So far, those which have been most effective have caused a partitioning between sugar and fiber resulting in increased sugar production at the expense of fiber. It should be pointed out that these studies followed directly from and are a result of the fundamental work on translocation carried out in Hawaii, emphasizing the theoretical basis for success if the right chemical were found to produce the ripening effect. This speculation was shown to be the case when the first active compound, Trysben, was shown to be a practical ripening agent. For a number of reasons, however, this material could not be registered. A subsequent search for compounds showed the activity of materials such as Cycocel, Hydrothol, isoaureoycin, valeric acid, several quaternary compounds -- eventually resulting in the registration of the first commercial sugarcane ripener, glyphosine, known by its tradename of Polaris. Within the last few years, this compound has been replaced by its mono-substituted relative, glyphosate, whose tradename is Polado.

An increasingly active and important field is that of herbicidal antidotes (also referred to as antagonists, safeners, or protectants), thought by many as "a new era of selective chemical weed control." These compounds are applied to the crop, usually to the seed, to make it resistant to the herbicide. Then the herbicide can be applied to the row and kill only the weeds.

An exciting area of current research and field application studies is the second generation gibberellin biosynthesis inhibitors (tetcyclacis, flurprimidol, paclobutrazol, and others). Results with these compounds indicate wide potential, not only for tree crops, but for rice production, grass seed production, control of vegetation along highways and rights-of-way, and production of various horticultural and agronomic crops.

Plant growth regulators also are suggested for overcoming environmental limitations such as resistance to drought, salinity and cold. This has been shown to be the case with gibberellic acid in sugarcane where the effect can only be achieved when applied during the winter months when there is normally very poor elongation of the sugarcane stalks. It is expected that considerble work will be done in this area during the next few years. Preliminary indications with materials such as Cycocel and other chemicals indicate that overcoming environmental limitations should be a rather fertile area for investigation. There are also indications that plant growth regulators may change resistance to disease and insect pests. The degree to which this can be turned to society's advantage has not been established, but will be the result of continued investigation in the immediate future.

Plant growth regulators have been used successfully to modify all phases of plant development, from seed to post-harvest behavior. Both yield and quality have been improved. In some cases risks and/or labor costs are reduced. In some cases the crop can be manipulated to fit the production system, timing, weather, and market demands. On the negative side, some important crops have yet to be successfully regulated by chemicals. The substances usually have short term effects rather than "life-long" effects. They must be applied with care, both as to timing and to concentration. In some crops only one stage has been successfully modified; however, in a few cases multiple growth regulation practices have been developed. The best examples of "seed to product" chemical management are sugarcane and apples. Success with these major crops holds open the possibility that chemicals can be found to successfully modify many important processes in most crops.

III. MAJOR BREAKTHROUGHS NEEDED

More basic knowledge is needed of whole plant physiology, biochemistry, development, and metabolism of target crops. This is especially true of the economic products involved or obtained from these crops, whether they be vegetative or reproductive.

Many crops are harvested long before the end of the potential growing season because of plant senescence. Therefore, a basic understanding of senescence in plants would go a long way in enabling investigators to be able to increase yields through control of this phenomenon. A series of Gordon Research Conferences has been conducted over the past several years on plant senescence. The progress made in slowing down senescence in a number of crops such as citrus through the use of plant growth regulators, particularly cytokinins, should serve as an impetus to increase the research efforts in this direction. An extension of this concept would be to understand how to extend the duration of growth of a specific organ such as a fruit or seed. This could have the net effect of increasing yields.

The development of specific screening techniques which are process-specific and often crop-specific will be necessary before additional major strides are made in the control of plant development through the use of chemicals.

As mentioned previously, because of the success with, the immediate economic use of, and the relative simple and straightforward methods of searching for herbicides, the weed-killing aspect of plant growth regulation has over-shadowed its other uses for the last several decades. Because the niches for uses of herbicides are rapidly becoming saturated, and, because plant growth regulators have been successfully introduced in some important crops, industry has increasingly turned its interest to the search for plant growth regulators within the last few years. However, it should be pointed out that the task of discovering a plant growth regulator is substantially more demanding than that involved in the search for a new herbicide. Estimates of the complexity and the costs connected with this complexity are estimated to be 10 to 1000 times greater than that for herbicide research. Nevertheless, the absence of effective plant growth regulators for most of the major crops makes this field extremely attractive -- with large potential payoffs. For success, long-term commitments are necessary, both to the research and development programs and to the time periods now necessary for approval by regulatory bodies.

Because of the long term commitment necessary as well as the high expense, much strategic thinking as well as gathering of information is needed for a plant growth regulator program. For example, in a screening program for herbicidal activity, one can screen a chemical and let the results of the screen suggest the crop or crops for which the chemical might be used for the control of which weeds. There is no screen such as this for plant growth regulator uses. First, one must decide the crop for which to develop a plant growth regulator. In reaching this decision, a considerable amount of market research information is necessary, covering such factors as: a) value of the crop, b) history of chemical use for the crop, c) what specifically one wants to do to the crop. Next, when the crop is chosen, one must know or should develop an extensive knowledge of the physiology and biochemistry of that crop. Next, one must answer how the plant growth regulator is to enhance the value of the crop after application. That is, is it to change some metabolic process, to change the physiognomy of the crop, or to increase yield *per se*? If the last, is the economic value of the crop in its vegetative or reproductive state? Is this value in a developing organ such as a fruit or in a root? Do you expect to apply the plant growth regulator as a foliar spray, directly to the soil, or through an irrigation system? To have its maximum economic effect, when should the chemical be applied? The complexities in searching for plant growth regulators for a given crop already have been amply demonstrated in studies with maize in which the need to determine the rate-limiting factor(s) and their interactions in order to determine when a plant growth regulator must be applied to have its maximum effect or, in some cases, to have any effect has been clearly shown.

There are other possible approaches -- more basic and/or more indirect. For example: (a) discover compounds with biological activity on plants and find crop situations where their effects would be useful; (b) learn the roles of natural hormones and devise ways to modify their effects, then find crop situations where these would be useful; (c) identify genes that control hormone activity and modify these genes to be receptive to specific growth regulating substances or inhibitors.

Such approaches may produce uses for crops of limited volume/value, but they are more likely to be used by public agency and university scientists than by private industry. However, progress in these areas will aid the more direct industrial approach described above.

Future chemicals must be synthesized which have higher biological activity, which have greater specific activity, and which have less mammalian toxicity. Both increases in biological activity as well as specific activity will help lower mammalian toxicity. Considerable progress has already been made in these areas.

A better understanding of the absorption and translocation of a given chemical in a given plant system will aid significantly in obtaining success with that chemical. An excellent example of the value of basic information on translocation is in the case of sugarcane where long series of studies aimed at an understanding of sucrose production and translocation in the cane plant paved the way directly for the success achieved in the ultimate discovery and use of ripeners to increase the yields in that industry significantly.

A number of obstacles exist in achieving the potential which is readily apparent for the use of plant growth regulating chemicals in agriculture. Not the least of these is the polarization in our country between government and industry. A fallout from this polarization affects two major areas of economic importance, one is regulations and the other is patent protection. The increasingly greater stress imposed by regulations -- and especially those pertaining to time constraints and to testing restrictions -- have particularly "stacked the deck" against small acreage crops and small value crops. These same regulations which require six to ten years from discovery for registration of a chemical for its use in agriculture leave a very short patent protection time for the company involved to retrieve its investment in the research necessary to discover and develop such a chemical.

An overall obstacle is the serious lack of funding of agricultural research, particularly plant agricultural research, compared to medicine. A fallout from this is obviously a scarcity of top notch students wanting to enter agricultural research because of financial considerations, both for funding of research programs and the existence of professional positions. Another obstacle to progress in the use of chemicals for the control of plant growth and development has been the lack of dedicated support to such programs. Only recently are plant growth regulator programs becoming removed as orphans of herbicide programs. When this removal is complete, proportionate progress should be attained. Another obstacle on a societal basis is the occasional need to set priorities based on reasons other than economic ones. As a society, many times there are overriding strategic reasons to develop a crop that can be grown within the confines of the U.S. borders. Our society needs some way to handle these strategic needs and not to rely exclusively on economics as the ultimate factor in all decisions. The last obstacle to be overcome is consumer acceptance. This can be accomplished by education, but such an effort is seriously restricted by the current attitude by some, who happen to be extremely vocal, that the use of chemicals for any reason and any purpose is bad.

IV. INSTITUTIONAL ARRANGEMENTS

Very clearly the order of input and effectiveness in research to develop plant growth regulation through the use of chemicals in descending order is private industry, followed by the federal government, followed by the state agricultural experiment stations and other universities with interests in plant biology. This is no doubt tied in to the obstacle mentioned in the previous section, that the overriding priority is based on economic reasons which gives the order of preference listed above. If strategic considerations were included, of whatever nature, I am sure these differences would tend to level out.

The relationship among these three groups, industry, federal government, and state institutions, is not at all equal. By far the poorest relationship exists between industry and the federal government with the possible exception of the U.S. Department of Agriculture. The relationship between the federal and state government is considerably better probably based on the states' dependence on federal funds for a substantial portion of their research funding. In general, quite good relationships exist between industry and state and private educational institutions. Even though these are the best of these relationships, they probably will still improve with the trend toward more financing of research by industry.

V. THE LIKELY TIMING OF COMMERCIAL INTRODUCTION

With the present regulatory climate, few introductions other than those already "in the pipeline" can be expected during the 1980's. Because of renewed interest in the use of plant growth regulating chemicals, we can expect a substantial number of new products or uses for existing products in the 1990's and before the year 2000 in spite of current restraints. Many of these will be extensions of uses of existing products because of the difficulty of registering new compounds. Many will be combination uses of existing products which now are used singly. There will also be a crossover usage. In other words, evaluation of chemicals used for one purpose but never evaluated for other purposes. Since so much of the basic work, the chemistry, the evaluation, as well as the expensive toxicology has been done, finding other uses for existing compounds, alone or in combination, might well have a major impact when compared to the introduction of new chemistry.

VI. POTENTIAL PRIMARY IMPACTS

There are a number of reasons why plant growth regulators will be more important in the future. First and foremost is that there will be savings in energy and costs. Second, more active molecules will be discovered resulting in less societal exposure. Third, advantage will be taken of naturally-occurring active molecules. When these are of sufficient activity, they will be used as such; when there is not sufficient activity to be commercially valuable, they will serve as models to develop closely related chemicals which will be active enough. Fourth, a re-examination will take place of pesticides which are already registered for other uses for their potential use as plant growth regulators. There are several examples which one can already refer to show this is a very fertile field. These include the cytokinin-like activity of the fungicide benlate, the abscission-inducing activity of the insecticide Sevin, and the antibiotic actidione. Sixth, will be the study of combinations of existing plant growth regulators for both additive and, hopefully, synergistic effects between them.

In the case of the use of chemicals to regulate growth and development of crop plants, yield increases must be of the order of ten percent or greater and the value to the consumer (farmer) usually has to be a return of $4.00 for every $1.00 invested. This can go up or down, of course, according to the consistency of the result obtained.

Plant growth regulators will give more ability to manipulate the timing of crops to fit the variability of weather, market demands, and production system constraints (labor availability, equipment, etc.).

Products used in the regulation of plant growth will either be new chemicals or new uses for old chemicals and particularly existing products as discussed in the previous section.

It should be emphasized that the use of plant growth regulators is one of the few serious positive contributions to agriculture since the introduction of breeding. Most of the other major uses such as irrigation, fertilizer, mechanization, are aimed at removing limitations rather than promoting to an apparent optimum.

Once they are available, the use of plant growth regulators will have an impact on the economic production of crops both because of their saving in energy and their saving in cash outlay. Increasing the yield of a crop for a given area of land means an incremental gain, the only output for which is the cost of the plant growth regulator and its cost of application. No extra land must be purchased, no extra land must be tilled, generally no extra fertilizer must be added, no extra weed control or other pest control measures must be taken, no additional harvesting need be made. There will be extra processing costs because there will either be additional plant material or additional economic product such as sugar, oil, or protein added to the crop by the proper use of the appropriate plant growth regulator.

As agricultural production practices become more intense, the potential for the use(s) of plant growth regulators increases. Europe is a good example. Its intensive agricultural practices has long warranted the extensive use of anti-lodging compounds on grain crops and the use of a variety of plant growth regulators on high-cost horticultural crops.

There are at least four plant product areas of a specific strategic value to the U.S. . These are (1) the development of a guayule rubber industry within the United States, (2) the increase in oleoresin in pine, thereby revitalizing the naval stores industry, (3) increased biomass in a number of crops or potential crops, and (4) the shortened time for the breeding of trees for forestry, particularly when combined with tissue and cell culture techniques now being developed in the biotechnology area. It will be necessary to utilize growth control techniques (including plant growth regulators) to control size of trees produced and propagated via biotechnology procedures in order to efficiently utilize "high density" orchards in the U.S.

REFERENCES

Agricultural Production Efficiency. 1975. National Academy of Sciences, Washington, D.C.

Archer, M.C., D. J. Hansen, and D. F. Loussaert. 1982. Corn yields -- rate-limiting factors and opportunities in plant growth regulation. In Chemical Manipulation of Crop Growth and Development (J. S. McLaren, ed.), pp. 253-265. Butterworths, London.

Avery, G. S., and E. F. Johnson. 1947. Hormones and Horticulture. McGraw-Hill, New York.

Johnson, D. G. 1980. The Politics of Food. The Chicago Council on Foreign Relations, Chicago.

Nickell, L. G. 1978. Plant growth regulators. Controlling biological behavior with chemicals. Chem. & Eng. News 56(41): 18-34.

Nickell, L. G. 1982. Plant Growth Regulators -- Agricultural Uses. Springer-Verlag, Berlin-Heidelberg-New York.

Nickell, L. G. 1982. Plant growth substances. Encyclop. Chem. Technol. (3rd ed.) 18: 1-23.

Nickell, L. G. 1983. Plant Growth Regulating Chemicals. Volumes I and II. CRC Press, Boca Raton, Florida.

Scott, T. K. 1979. Plant Regulation and World Agriculture. Plenum Press, New York.

Staples, R. C., and R. J. Kuhr. 1980. Linking Research to Crop Production. Plenum Press, New York.

Weaver, R. J. 1972. Plant Growth Substances in Agriculture. Freeman, San Francisco.

APPENDIX

Chemicals Mentioned in Text

Common Name	Trade Name(s)	Chemical Name
chlormequat chloride CCC	Cycocel	2-chloroethyltrimethyl ammonium chloride
CMU, monuron	Telvar	3,(4´-chlorophenyl)-1,1-dimethylurea
2,4-D	Weed-B-Gon, Plantgard, Agrotect	2,4-dichlorophenoxyacetic acid
DCMU, diuron	Karmex, Dynex	3-(3´,4´-dichlorophenyl)-1,1-dimethylurea
diquat	Reglone, Reglox	6,7-dihydrodipyridol (1,2-α:2´,1´-c)pyrazdiium dibromide
endothall	Hydrothol (Ripenthol)	7-oxabicyclo-(2,2,1)heptane-2,3-dicarboxylic acid, monoalkylamine salt
ethephon	Ethrel, Cepha	2-chloroethylphosphonic acid
gibberellic acid	Pro-Gibb, Gib-Sol, Gib-Tabs	2,4a,7-trihydroxy-1-methyl-8-methylenegibb-3-ene-1,10-carboxylic acid-1,4a-lactone
glyphosate	Polado	N-phosphonomethyl-glycine
glyphosine	Polaris	N,N-bis(phosphonomethyl)glycine
isoaureomycin	Isochlor tetracycline	---
IBA	Hormodin	3-indolebutyric acid
maleic hydrazide	MH-30, Sprout Stop	1,2-dihydro-3,6-dipyridazinedione
MCPA	Agroxone, Ded-Weed	4-chloro-2-methylphenoxyacetic acid

.../

NAA	Tre-Hold, Fruitone-N, Transplantone	1-naphthaleneacetic acid
paclobutrazol	---	1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)pentan-3-ol
paraquat	Gramoxone	1,1′dimethyl-4,4′-bypyridinium dichloride
2,3,6-TBA	Trysben	2,3,6-trichlorobenzoic acid
tetcyclacis	---	5-(4-chlorophenyl)-3,4,5,9,10-pentaaza-tetracyclo-dodeca-3,9-dione
n-Valeric acid	---	n-pentanoic acid

Background Paper No. 12

Plant Disease and Nematode Control

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PLANT DISEASE AND NEMATODE CONTROL

Summary

Plant diseases are caused by viruses, fungi, bacteria, nematodes, and other microorganisms. Collectively, these organisms cause losses before and after harvest estimated at $18.6 billion annually. Only a few of the thousands of species of pathogens and insects cause concern, however; the rest are controlled by natural immunity. Many organisms that do cause loss may theoretically be controlled by managing more wisely the mechanism of host-plant resistance. This area is a major one for research.

Some beneficial microorganisms help protect plants from disease. In addition to their nutritional benefits, nodulating bacterial and mycorrhizal (root-extending) fungi render some plants more disease resistant. Microorganisms also provide a vast gene pool for improving plants and other microorganisms through recombinant DNA technology. That technology is already available for synthesizing microbes of naturally occurring products for use as pesticides. Such genetic engineering should lead to new biocontrol agents; for example, modified plant viruses that will give cross protection. One success story is that of crown gall, a serious bacterial disease of many woody and herbaceous plants. Crown gall is now controlled biologically by the K84 strain of bacterium that is a close relative of the bacterium that causes the disease. Inoculating a seed or transplant with K84 produces a bacteriocin that protects against crown gall.

Other examples of biocontrol include using disease-suppressive soils and pasteurizing the soil to kill pathogens but not thermophilic beneficial microbes. In addition, some cultural practices (fertilization, irrigation, and stubble and debris management) can be

refined to effect biocontrol. For example, continuous cropping can be used to allow antagonists to pathogens to increase, as in the control of potato scab.

Disease-resistant cultivars can be bred and resistance managed. Genetically, plant resistance is conditioned by major-effect genes and minor-effect genes. Although major-effect genes are easier to work with and give more dramatic results, their effectiveness in the field has frequently been disappointing. Thus researchers have turned to minor-effect genes, which are more difficult to work with but are the most successful way of controlling disease in the homogeneous cultivars demanded by mechanized Western agriculture. Major-effect genes show promise for controlling disease in heterogeneous cultivars, as occurred with multiline oat and wheat cultivars developed in Iowa and Washington. Even highly epidemic foliar pathogens can be controlled in this manner. A major-line of research may result in using resistance genes to obtain diversity without sacrificing bona fide needs (as opposed to merely cosmetic needs) for uniformity. This may be one of the fastest ways simultaneously to control certain highly epidemic diseases and to reap the tremendous potential benefits from plant genetic engineering.

Additional work is needed at all levels of pesticide development, but is especially needed to complete development of systemic pesticides that have two sites of activity on the molecule, thereby extending the pesticide's effective life. Other research is needed on more effective delivery systems for systemic pesticides.

Other research will be directed to developing naturally occurring chemicals that will stimulate the plant's defense mechanisms or enhance activity by biocontrol agents. Ultra-low-volume delivery systems will be needed for these and regular pesticides that are active at very low dosages.

A final important area for research is that of crop loss assessment. Although it is possible to assess plant loss from single pathogens, weeds, and arthropods (and a few combinations of these), such assessments are less precise when made for larger areas, several cultivars, and a wide variety of plant stresses. Research to improve crop loss assessment will help set research priorities and aid in making management decisions.

PLANT DISEASE AND NEMATODE CONTROL

J. Artie Browning

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Plant Disease & Nematode Control

J. Artie Browning[1]

I. Introduction

A. Economic Necessity for Plant Disease and Nematode Control

In the most detailed study ever made of yield losses from plant diseases in the principal crops of an entire state, Main et al. (1984) estimated that plant diseases and nematodes caused reductions in yield and quality of $508 million (including the cost of control measures applied) in North Carolina's 1983 crops valued at $6.7 billion. If all 50 states experienced reductions from 1983 crops proportional to those estimated for North Carolina, they would total ca. $18.6 billion loss for the nation. That 7.6% reduction in value is considered modest. It emphasizes the need for still more accurate estimates and for a National Crop Loss Assessment System. Also, it points out that disease control is necessary if agricultural production is to be stabilized at a high level. This paper considers control options to help make that possible.

B. Definition and Scope

"Control" as used herein means to manage disease-causing microorganisms (bacteria, fungi, viruses, nematodes, etc.) so they do not prevent a cultivar from yielding to its genetic agronomic, horticultural, or silvicultural potential.

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The management technology used should include everything in the repertoire of the team of contemporary pathologists, breeders, and other plant scientists: quarantines; disease-free planting material; resistance/virulence gene management; genetic engineering; development, deployment, and prudent use of superior fungicides, nematicides, and bacteriocides; and the discovery, development, delivery, and management of biocontrol agents.

The objectives are to effect lasting control of present pathogens and to minimize the probability that unknown or unimportant indigenous microorganism strains can increase to pest status or that exotic pathogens will be introduced and become established. Achieving thses objectives becomes far more probable if a holistic approach to control is taken, i.e., sharing the work-load of management broadly and not asking any one gene, pesticide, or technology to bear it alone; too frequently a pathogen has proven capable of overcoming a control measure or management tactic used singly. The result of this holistic approach should be a well-buffered, biologically rich agricultural ecosystem (agroecosystem) in which the protection a given cultivar enjoys is not a characteristic just of each individual plant, as in the past, but is a product of the entire population of plants, beneficial microorganisms, and cultural system. Even so, during periods when the environment is especially favorable for disease development, pesticides may be needed to improve yield and/or quality and sustain them at a high level. If so, their application should be based on action thresholds and a predictive system to guide timing and to give a reliable indication of need. Pesticides and resistance are mutually complementary; the more host-plant resistance a cultivar possesses, e. g., the less pesticide should be required to effect control. Also, the potential effect of pesticides on non-target microorganisms must be considered. If *genetic* host plant resistance can be managed wisely to achieve *epidemiologic* dilatory[2] resistance, and if cultural practices (e.g., seed inoculation, crop rotation, or intercropping) can be maintained or developed to maximize effect of biocontrol agents, little of the work of disease management should remain for non-biological disease-management agents in the sustained yield agroecosystems (SYAEs) of the future. *SYAEs must be the long term goal of USA and world agriculture*.

The above philosophy calls for a large repertoire of measures and/or tactics to be available for managing a given disease, although only a subset from that repertoire might be needed in a given case. This philosophy of managing a *single* disease, applied also to the management of other pests -- pathogens, nematodes, arthropods, and weeds -- becomes integrated pest management (IPM). IPM was introduced and fostered by the entomologists to reduce insect damage while ensuring responsible pesticide usage and helping protect the environment from toxic substances. IPM is a powerful paradigm and a necessary strategy toward a still broader concept of "plant health".

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[2] Dilatory resistance expresses an epidemiologic concept for a host plant resistance that, however inherited, delays the rate of development of a pathogen.

Plant health, as used herein, is far more than the absence of plant disease. Plant health describes the relative freedom of the green plant and its ecosystem from the effects of biotic and abiotic sources of stress that limit the attainment of their maximum genetic potential over time. Obviously, this concept of plant health considers and attempts to unify in a holistic program all causes of plant stress -- pathogens, nematodes, weeds, insects and other arthropods, water and nutrient excesses and deficiencies, soil pH, salinity, etc. -- and concerns all the disciplines responsible for studying them and their effect on the green plant.

This paper will concentrate on the control of plant diseases with reference to the broader concept of plant health as indicated, primarily in Section IV, "Institutional Arrangements".

II. Major Lines of Research

A. Develop the Science of Crop Loss Assessment

Minimizing losses from plant diseases is the raison d'être of phytopathology. Yet only recently has the necessity of assessing losses with accuracy and credibility taken on respectability in the USA. More reliable information of the loss sustained by crops from different stress causes is needed 1) to help set research priorities and 2) to aid management decisions. This requires first research to develop crop loss assessment as a science, and then the application of that science across the whole nation. This justifies a high priority as it is central to assigning other research priorities.

Further, crop loss assessment is necessary not only to help prioritize among the many research needs in plant pathology, but to help obtain funding to meet those needs in competition with other demands for funds. As the science and art of crop loss assessment mature, they should be incorporated into a holistic national system.

Disease symptoms vary from being striking if caused by foliar pathogens, to escaping notice if caused by "root nibblers", to death of the plant. Diseases cause yield losses that are especially difficult to assess for they result from very complex physiological reactions that occur over time well after the different times infection is initiated, and that vary in rate and extent of development with different cultivars and with an ever-changing environment. In contrast, losses from arthropods and weeds correlate better with pest numbers that are more readily seen and sampled. Even if loss can be assessed with some accuracy in experimental plots using pesticide-protected checks and/or resistant and susceptible isogenic lines, a great reduction in precision occurs with projections over different cultivars and whole counties, states, and agricultural belts. Agriculture deserves better.

B. Cropping Systems, Agroecosystem Components, and Interactions Between and Among Them

1. The agroecosystem[3] -- the unit for research as for commercial production

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[3] Whenever agroecosystem is used in this paper, generally horticultural and silvicultural ecosystems are implied as well.

Only in a test tube, petri dish, or in gnotobiotic culture does an organism grow in isolation; in nature and agriculture, each organism shares an ecosystem with many other higher plants and animals and microflora and microfauna. Some of these agroecosystem components are deleterious (pathogens, insect, or weed pests) to the plant and/or animal species favored by man; others are beneficial. Most work by scientists has been on one component of the agroecosystem at a time -- nitrate nitrogen, Rhizobium, a perennial weed, or a given pathogen or insect on one crop, e.g. -- as if other components of the agroecosystem didn't exist and didn't interact. But they do exist and interact -- some positively, i.e., favoring man, and some negatively. It is a thesis of this chapter that the agroecosystem should be the basic unit for research just as it is the basic unit for production. Probably few activities could benefit agriculture more dramatically than researching interactions among the many agroecosystem components and adopting those that are positively synergistic -- i.e., a combination of components that interact in a positive way to result in higher yield and/or quality from the agroecosystem and that portend doing so on a sustaining basis. Such synergistic components would result in an agroecosystem that would be much greater than the sum of its parts -- a phenomenon to be sought by agricultural scientists and if, found, prized by the agricultural community and the nation.

Scientists have done an excellent job of studying the agroecosystem components. With rare exceptions, however, no one integrates them short of the farmer himself, and he hasn't the research training, statistical tools, venture capital, or interdisciplinary collaboration necessary to find the best of the many combinations of possible interactions among agroecosystem components.

Further, an agroecosystem unit is not necessarily just what it may seem in a single year. In a given agroecosystem unit, each crop may compete for or augment use of the land, and each may help solve or complicate pathogen and other problems in the agroecosystem unit. For example, in much of the Corn Belt, corn and soybeans share the ecosystem unit; in Kentucky, winter wheat and soybeans; on the Coastal Plain of Southeast Texas, rice and soybeans; in the Texas Blacklands, cotton and grain sorghum or winter wheat grazed early on by beef cattle that constitute part of the agroecosystem's production. In Texas, a Rhizoctonia solani strain attacks both rice and soybeans and becomes part of the management problem and a research objective.

We must develop cultivars and holistic SYAEs in which to grow them to maintain crop yield at a high level over time. Soil fumigation studies show that most major USA crops as grown today (i.e., in monoculture or 2-year rotation, frequently with reduced tillage) often yield only 50-75% of genetic potential as limited by climate, soil, and agronomic inputs. We cannot and should not advocate fumigating soil over broad areas, but such studies show the central importance of healthy roots. SYAE research must find a way to achieve the increased growth response that follows soil fumigation, but without the expense and environmental risk of using soil fumigation over extensive areas. The answer to this demonstrated need for root health, and disease management for the rest of the crop plant and its agroecosystem, may come from any or a combination of the major lines of research that follow.

2. Breed cultivars to effect epidemiologic dilatory resistance and reduce the rate of spread of the pathogen

With plant agriculture, the choice of a cultivar is probably the most important single choice a grower makes. This is true whether the grower is planting a long-term orchard or forest, or a 100-day wheat cultivar that can be changed next year. The cultivar must be adapted well to the soil type and to the average climatic conditions likely to be encountered. Also, it becomes such a dominant component of the ecosystem that it largely determines the microflora and microfauna that it selects for or against, both above and below ground. It may select for (favor) microorganisms that are beneficial as well as some -- pathogens -- that are detrimental. These pathogens interact among themselves, with the beneficial microorganisms, with the environment, and with the cultivar, weeds, and other crop plants in the rotation.

For pathogens in the agroecosystem, it is clear that one trend seems to be the goal of many plant pathologists and breeders: select a cultivar that reduces the rate of development and spread of prevalent strains of pathogens. Rate reduction is an epidemiologic concept for which the term "dilatory resistance" has been coined. There is a vast literature on such "resistance theory". Only a minimum follows:

a. Breed and deploy genetically homogeneous cultivars that effect dilatory resistance. It is commonly considered that, genetically, there are two basically different types of host-plant resistance, one conditioned by major-effect genes and one conditioned by minor-effect genes. Barring linkage, these are completely miscible and, if possible, should be used in combination. The major-effect genes are easier to work with, give more dramatic effects, and therefore were used first and widely. The effects of such genes in many crops have frequently proven ephemeral, however. Therefore, for the past 10-15 years, there has been a strong tendency to discard them from breeding programs. Thus, the minor-effect genes, which are more difficult to work with, have been those in greater demand by many pathologist-breeder teams that desired lasting or "durable" resistance. Indeed, the use of minor-effect genes seems the surest way of achieving the highly desirable rate-reducing dilatory resistance (slow rusting, etc.) with the homogeneous cultivars generally demanded by mechanized western-type agriculture. The same epidemiologic result can be achieved with heterogeneous cultivars and major-effect genes, however, and genetic engineering may stimulate their use.

b. Breed and deploy heterogeneous cultivars that effect dilatory resistance. Another crop-management strategy for achieving the same epidemiologic goal -- dilatory resistance -- is to grow cultivars that are diverse for major-effect genes. This has not been accepted to any extent in the USA, largely, for field crops, because agriculturists are enamored with the paradigm of uniformity for uniformity's sake. There are many bonafide reasons for uniformity (mainly planting and maturity dates, height, and certain quality traits required for mechanized agriculture). But when unneeded uniformity becomes cosmetic and endangers rather than protects a crop from diseases, alternatives should be considered. The alternative of managing heterogeneous cultivars to effect disease control was proven with multiline oat cultivars developed in Iowa, with multilines of wheat in Washington, India, The Netherlands, and Colombia, and with 3-way cultivar mixtures of barley in England. If a pathogen is a sufficient threat, if managing homogeneous resistance does not effect control, and if major-effect genes are available, this is a proven way of controlling even highly epidemic foliar pathogens. The strategy has also been shown effective against nematodes such as the soybean cyst nematode in Illinois, insects such as the wheat Hessian fly in California, and relatively unspecific pathogens such as wheat Septoria in Wales. Therefore, a major line of research should be to learn to manage resistance genes in the field so as to obtain the many potential advantages of diversity without compromising the bonafide needs for uniformity required for mechanized agriculture.

This becomes especially relevant at this time and is given special emphasis herein because of the potential of genetic engineering. Transfer of major-effect resistance genes may be the most important contribution of plant genetic engineering to agriculture. But there seems little point in using the most modern technology to transfer such genes without the follow through to actually manage them more successfully in the field. That is the goal of research proposed in this section.

3. Discover or develop, deploy, and learn to manage biocontrol agents in the agroecosystem

Most biocontrol agents of plant pathogens are microorganisms, and they may function as hyperparasites, inhibitors, competitors, or some other form of antagonist. The creation of novel biocontrol agents by the genetic engineering technology appropriate to microorganisms is an exciting possibility. Biocontrol is especially promising, although relatively undeveloped and under-utilized, for use in commercial greenhouses or aquaculture. In the field, a hairless root system is of little value to the plant, but beneficial bacteria, e.g. Rhizobacteria, can enable a plant to grow as if it had received fertilizer. Indeed, much expensive fertilizer and irrigation water have been applied unwittingly to compensate for poor root health. This was demonstrated by bacterization of seed and by fumigation of soil and shows clearly the potential benefits of root health via bacterization and other means of biological control. There is a fast-growing interest in biological seed treatments with organisms that colonize the rhizosphere and protect rootlets and root hairs from premature death from Pythium and other pathogens.

With all the promise of seed treatment with biocontrol agents, delivery of biocontrol agents to solve soil-borne disease problems remains a major problem that should involve agriculture engineers, chemists, etc. in possible solutions. Selection of biocontrol agents for soilborne pathogens will be aided by the careful study of the rhizosphere environment. Root exudates are under genetic control and they influence which microorganisms occur in the rhizosphere, including a possible protective microflora and microfauna. Selection for root exudates to encourage a protective microflora and microfauna should be a breeding objective. Basic studies concerning soil microbes and their interactions with their environment are prerequisite to successful biocontrol. If a fit biocontrol agent can be introduced to a rhizosphere successfully, or an indigenous agent encouraged, its effectiveness may be maintained a long time.

For foliar pathogens, new biocontrol agents are needed together with predictive systems to guide their use. New agents have been developed through genetic engineering but the courts have prevented their use. Once they are cleared for use, the natural laws of genetics and of epidemiology will govern their relationship to their host and to the environment as for other organisms, and these laws must be discerned. Probably biocontrol agents will have to be applied to foliar pathogens as needed. Once released into the environment, they are unlikely to survive for long periods in numbers sufficient to effect biocontrol.

4. Maximize benefit to the agroecosystem of mycorrhizal fungi, nodulating bacteria, and other beneficial microorganisms

Economic benefits and liabilities of the nitrogen-fixing bacterium Rhizobium on legumes in a crop rotation are well known. Efforts are underway to use genetic engineering or other means to obtain nodulating grasses (corn, wheat, etc.), possibly with the bacterium Sprillum spp., and avoid both artificially applied N and crop rotation. Whether this is feasible may depend not just on scientific breakthroughs, but on the energetics of the plant's use of photosynthate to fix nitrogen rather than filling seed with carbohydrate.

Less well known is that nodulating bacteria and mycorrhizal fungi influence the plant's response to disease. Mycorrhizal fungi are "root extenders" which increase the plant's uptake of phosphorus, other nutrients, and water by plants. There are many reports of their rendering a plant less damaged by plant nematodes and other soil-borne pathogens. There are also a few reports of their increasing susceptibility. Several foliar pathogens can attack mycorrhizal plants more severely than they attack non-mycorrhizal plants. Likely this is because of the faster growth rate and increased leaf canopy from improved plant nutrition of mycorrhizal plants. "The obvious approach with mycorrhizal fungi should be to encourage their presence on plant roots whenever needed to help the plant obtain phosphorus or other nutrients, or whenever benefits can be demonstrated but not duplicated by simply adding fertilizer. If plants with VA mycorrhizal associations are more susceptible to certain diseases (e.g., verticillium wilt, rust, mildew), this is not justification for elimination of the mycorrhizae. Our approach should be to provide conditions for vigorous, healthy plant growth; such plants will thereby have a higher level of general resistance to pathogens favored by a stressed host, and for those diseases that may increase in severity, some other method of disease control can be found" (Cook and Baker, 1983). Clearly, additional research is justified on nitrogen-fixing organisms, mycorrhizal fungi, and other beneficial microorganisms. But, especially, research is indicated on interactions among these and other components of the agroecosystem, including pathogens.

5. Cropping systems and interactions among them and ecosystem components

I have mentioned many ecosystem components: host crop species and cultivars in monoculture or various patterns of diversity in space or time; deleterious pathogen, insect, and weed pests; natural pest enemies that effect biological control; and other beneficial microorganisms including nitrogen-fixing bacteria and mycorrhizal fungi. To these must be added the physical components of the environment such as amended by man, as pesticides, fertilizer components, irrigation, etc., and other cultural practices such as planting time and depth, row spacing, and the tillage system.

Probably many unknown positive or synergistic interactions are lurking in the above patchwork that could be elucidated for the benefit of agriculture with complex systems research. Our main concern, however, is control of plant diseases, including those caused by nematodes. We know of many interactions: For example, herbicides render sugarbeets more susceptible to root rots. Mycorrhizal fungi aid phosphorus uptake; addition of phosphorus fertilizer or infection by mycorrhizal fungi make the vigorously growing wheat more susceptible to rust or powdery mildew, which, in turn, must be controlled by field management of resistance genes or other means. Controlling moisture and fertility to minimize plant stress makes wheat more resistant to Fusarium root rot.

Some plant pathogens, especially viruses, may interact to result in more or less severe disease. The latter may even give induced resistance and be a means of biocontrol. Weeds may not just compete with crop plants but be allelopathic to them, as with Canada thistle, quack grass, and walnut. Weeds also may be alternate hosts to pathogens and biocontrol agents and enable insect pests and their natural enemies to survive in the ecosystem. Either may serve as virus reservoirs. Pathogens can complicate insect-management decisions and vice versa.

Doubtless there are many potential interactions among the large number of biotic and abiotic components of the agroecosystem. The farmer cannot avoid being a great integrator, but he cannot evaluate the many possible combinations of ecosystem components available to him. This must be done by the agricultural researcher. Research must identify candidate components, learn to manage them, and then further refine the system for use by growers who can realize the synergistic potential in his fields. With a mature, highly developed agriculture, large yield increases may come mostly from such interaction effects.

6. Population genetics/biology of ecosystem components

> Dogs have fleas
> And fleas have fleas
> On their backs to bite them.
> And little fleas
> Have lesser fleas,
> And so ad infinitum.

This well known ditty expresses well the philosophy of biological control, omitting only man's intervening to fine tune the natural process in his favor. It also expresses the starting point of host-parasite population biology/genetics. Since H. H. Flor showed over four decades ago that the disease expression between a host and a specific pathogen is controlled by complimentary genes in host and pathogen, scientists have found the gene-for-gene hypothesis to explain a high percentage of the variability reported in the literature between all classes of hosts, including man, and all groups of pathogens.

Thus, gene-for-gene relationships probably govern not only the host-pathogen relationships that are our major concern in this chapter, but the relationships between the plant pathogen and its pathogens, and even pathogens of the latter, etc. Also, pathogens of the pathogen's antagonists, etc. are of concern.

A vast literature exists on crop cultivars and their virulent and avirulent pathogenic races, but population genetics/biology research is scant that really tracks gene flow in the host and pathogen populations. This must be done, not only to refine crop management schemes for actual use, but to resolve theoretical aspects of major- and minor-effect genes and their conservation and use.

This type of research is needed not just for understanding interacting populations of hosts and pathogens within a given field, which commonly are the main concern of the pathologist-breeder team, but of all interactions in the agroecosystem unit -- between and among crop and weed species; between the cultivated crop and the fence-row interface; between crop species being intercropped or rotated; within maize that has tropical pathogens in common from Central America and Mexico to Texas and, rarely, depending on environment, on to the Corn Belt; or within wheat that is virtually continuous from Mexico and Texas on into the Prairie Provinces of Canada. Each of these examples describes a single geographical pathogen distribution system.

The same laws of population genetics/biology and of epidemiology must apply to a plant pathogen that becomes a host to its pathogen -- a mycoparasite, e.g. Thus, population geneticists can also contribute much needed understanding to interactions among all classes of microorganisms in the agroecosystem.

To summarize this section, population genetics/biology research is needed to give genetic explanation to many of the interactions included above under III. B., "Cropping Systems, Agroecosystem Components, and Interactions Between and Among Them".

C. Genetic Engineering

1. Recombinant DNA technology

The first impact of genetic engineering on plant agriculture, after an explosion of basic knowledge on all aspects of genetics, biochemistry, and cell and developmental biology, may be in the biological synthesis by microbes of naturally occurring products for potential use as pesticides. The technology is now available for this. As the nutrient medium and physical environment for biological synthesis are carefully controlled, this requires only the transfer of the gene responsible for the product desired into the synthesizing organism, probably a bacterium.

It is important to emphasize that microorganisms represent a vast gene pool for improving plants and other microorganisms, just as plants are a vast gene pool for improving other plants. Hence, genetic engineering should lead to the development of new biocontrol agents, e.g., modified plant viruses to give cross protection, or bacteria for introduction into the root environment via seed treatment. Such agents may prove a source of genes to improve higher plants.

It is commonly stated that the greatest pay-off from genetic engineering of plants may be in the area of disease resistance. Using recombinant DNA technology, major-effect resistance genes may be transferred among species -- e.g., from a giant Sequoia or a microorganism to rice or wheat. It should be possible, using the techniques of genetic engineering and monoclonal antibody technology, to learn the key gene products and to transfer major-effect genes among different species relatively soon, but there is no reason to expect that groups of minor-effect genes will be transferred readily or soon. It becomes especially important, therefore, to develop the technology and accept the paradigms necessary to manage major-effect genes wisely in the field (as in Section II.B.2.b. above) to extend the useful life of the genetic material. Crop management as in the past portends a useful life for a major-effect gene of only 3-5 years -- a short payoff for investors and for an expectant, hungry world. Good genes are too valuable to squander thusly. The valuable, patentable genes from genetic engineering will be even more so.

A caveat is necessary. I mentioned that the environment is carefully controlled for biological synthesis and made no mention of fitness. Quite the opposite is true of new, genetically engineered cultivars or biocontrol agents to be used in the field. Their success will depend as much on fitness as on the target gene incorporated. To maximize the chances that the released organism possesses all attributes required for successful deployment, it must be subjected to rigorous testing of the type that is characteristic of most breeding-pathology testing programs.

2. Protoplast and meristem culture. Plants regenerated from protoplast cultures obtained from somatic tissue have shown great variability, including variation for disease resistance. The nature of this is not fully understood. This merits intensive research and, if possible, making this source of variability available to pathologist-breeder teams. Meristem culture followed by sanitation has been very successful in the control of viruses systemic in vegetatively propagated cultivars.

D. Bacteriocides, Fungicides, Nematicides, and Other Chemicals

Seek new, improved types of each of these pesticides. This should include types that are absorbed differentially by the target organism and that have curative (eradicative) activity. Develop formulations for slow, controlled release.

For fungicides, systemic products with highly specific modes of action seem to be overcome by pathogens just as pathogens quickly overcome many highly specific major-effect genes used singly. Two or more sites of activity on the molecule, or management strategies incorporating the diversity principle, may help. We need fungicides effective against soil-borne pathogens that, like the herbicide glyphosate, can be applied to the foliage and that then will translocate basipetally -- but without killing the plant, as glyphosate does. Possibly the new fungicide, effective at ultra-low dosages, could move basipetally following seed application at planting. In either case, the new fungicide should move into the roots and possibly the rhizosphere to inhibit the attacking pathogen. This requires, first, research into the physiological nature of compounds with basipetal translocation capability and control mechanisms for this vital process.

Also, much more research is needed on other aspects of systemic fungicides including secondary plant products and their relation to plant growth regulators. A special need is for systemics that, after foliar application or injection into the trunk, will translocate in trees and kill an invading pathogen, such as the fungi that cause Dutch elm disease, live oak wilt, fusiform or white pine blister rust, or Verticillium wilt of pistachio. First, basic research may have to be done on anatomical and physiological constraints to translocation in trees.

Good, economical plant disease bacteriocides have been rare relative to fungicides. Much additional R&D is indicated for them, some of which would parallel that for fungicides.

For nematicides, especially with the recent demise of DBCP (dibromochloropropene), EDB (ethylene dibromide), and DD (mixture of dichloropropane and dichloropropene) from EPA rulings and corporate decisions, there is great need for R&D of effective, environmentally safe products.

Also, research new means of application, including carriers (possibly vegetable oils) that facilitate using ultra low volume systems. It is important to study very small droplet sprays for distribution and drift pattern, field reentry time, and other potential health hazards. Research and develop new target application systems such as wick applicators and especially electrostatic sprayers.

With imaginative research, it may be possible to develop other chemicals for use in disease management, possibly through natural-products chemistry. These could be novel combinations of plant or microorganism growth regulators that are highly specific for a plant, a pathogen, or a biocontrol agent in extremely small amounts not unlike pheromones and insects. These might stimulate the plant's own natural defenses, enhance activity of populations of biocontrol agents, and/or in some way disadvantage the pathogen population.

III. Current State of the Art

A. Crop loss assessment

Crop loss assessment is now possible in experimental plots for single pathogens, weeds, and arthropods and, in a few cases, for combinations of certain of these pests. But loss estimates lose precision greatly when they are projected from field plots to large areas and to additional cultivars and interacting biotic and abiotic sources of stress. That loss assessments can be handled now at an acceptable if marginal level of confidence using a computer-assisted system is illustrated by Main et al. (1984). They included a table for each crop with a competence statement for each disease estimate, viz., C=confident, R=reliable, and I=indicative. Presently few R's and fewer C's are listed. The challenge now is to develop crop loss assessment as a quantitative science that can greatly increase the numbers of R's and C's.

B. Cropping-System and Agroecosystem-Component Interactions

1. Development of SYAEs

Little, if any, work is being done to develop SYAEs. Conservation and/or minimum tillage and inter- and multiple-cropping-systems research touch aspects of the SYAE concept. Considerable "maximum yield" research is being done; of course, with the addition of "over time" this would be SYAE research, but that reflects a significant philosophical difference. Possibly the closest approach to SYAE research is the renewable or "regenerative agriculture" program at the Rodale Research Center, Emmaus, Pennsylvania. This, too, differs some in concept; sustainable is not the same as regenerating, possibly after exploiting, sometimes repeatedly, in response to market conditions. Regenerative agriculture may represent the closest approach to a SYAE that is practical in the real world, however.

2. Breeding cultivars to effect epidemiologic dilatory resistance, whatever the means of genetic control

Slow progress is being made. Oligogenic resistance conditioned by major-effect genes is easiest to breed for but frequently has proven ephemeral in use. Consequently, it has been discarded wholesale from breeding programs for some crops in favor of resistance conditioned by minor-effect genes. Efforts to use minor-effect genes are continuing, with considerable success. Clearly, in many cases, genes for several polygenically controlled systems (e.g., yield, height, quality, resistance to each of several diseases or arthropods) are gradually being combined successfully, and the effort to do this should continue. But meantime major-effect genes are too valuable a natural resource to discard. They can augment polygenic resistance in homogeneous cultivars, and they can serve well in diverse populations. The two resistance types can serve best with minor-effect genes in the background of cultivars diverse for major-gene resistance. The only problem with doing this is a paradigmatic one, of breeder-pathologist teams enamored with the paradigm of uniformity for uniformity's sake. But further work is being done in Illinois, Iowa, Minnesota, Texas, and Washington states in addition to considerable work overseas to make this proven field-management technique operational. The need to be able to manage major-effect genes well in the field after they are incorporated by genetic engineering into cultivars for commercial production is a great impetus to perfecting our field-management capability of major-effect genes.

3. Biocontrol agents

Several examples must be given to illustrate the current state of the art for biocontrol.

Heterobasidion (Fomes) root rot of pine formerly was one of the most destructive pathogens of managed pine stands. Spores of the pathogen, Heterobasidion annosum, germinate on freshly cut stumps and the fungus grows through root grafts to standing timber. But a fungal antagonist, Peniophora gigantea, whose spores are applied to the freshly cut stump, will preempt infection by H. annosum, a poor competitor. P. gigantea spores can be applied in aqueous suspension or even in the lubricating oil of the chain saw. P. gigantea was the first antagonist registered by EPA for the biocontrol of a plant pathogen in the USA.

Crown gall is a serious bacterial disease of many families of woody and herbacious plants. It is amenable to biological control by strain K84 of a bacterium that is a close relative of the pathogen. If seed or a transplant is first inoculated with a suspension of K84, infection sites are occupied by K84 that produces a bacteriocin and protects the host from becoming infected by the pathogen. This is the most widely used biocontrol of a plant pathogen by an artificially applied antagonist.

The chestnut-blight fungus was introduced into the USA from Asia in 1904 and later into Europe. It decimated chestnuts above ground in eastern hardwood forests. Fortunately, chestnuts continue to sprout from stumps, indicating that germplasm of this valuable species is still available and waiting for a control measure to allow it to express itself again. Hypovirulent strains of the fungus, caused by infection by a double stranded RNA virus-like agent, have been used successfully in Europe for biocontrol. The agent both reduces rate of fungal spread and enables the tree to heal old cankers. Hypovirulent strains occur in the USA also and they are being researched. Thus far, however, natural spread of the virus-like agent has been more restricted in USA than European forests because of many more mating types of the fungus in this country. These limit natural spread of the agent by hyphal fusion. Artificial delivery of this biocontrol agent is indicated for the USA.

A novel use of biocontrol involves ice-nucleation-active (INA) bacteria. These nonparasitic exopathogens do not cause disease directly. But by nucleating ice between -2 and -5°C, they facilitate frost injury of plants and then utilize the resultant food source. The INA bacteria can be controlled with a bacteriocide, but this must be four or more days prior to frost, otherwise the corpuses of INA bacteria still will be effective ice nucleators. The INA bacteria can be displaced by competitive, naturally occurring non-INA strains of the same bacteria. Such strains have been developed by genetic engineering but courts have blocked field tests.

Many other examples of biological control are less dramatic or well understood but may be just as effective. Many of these involve soil-borne diseases and cultural practices. Sometimes biocontrol is effected by continuous cropping to allow antagonists to increase, as in potato scab or take-all of wheat. Trichoderma may be added to soil artificially where it is a very effective mycoparasite. Trichoderma inoculum is available commercially for this purpose. Soil may be heated with aereated steam or, covered with clear plastic, by the sun (solarization) to kill pathogens and favor thermophyllic antagonists that buffer against reinfestation by pathogenic microorganisms. Naturally disease suppressive soils may be used. Cultural practices (e.g., fertility, irrigation, stubble and debris management) may be refined to favor biocontrol and, therefore, the host. Combinations of the above have tremendous potential for disease management. Modeling should aid understanding of interactions involved. Developing delivery systems for biocontrol agents, probably on the seed, will be a major challenge.

4. Maximize benefit to the agroecosystem of mycorrhizal fungi, nodulating bacteria, and other beneficial microorganisms

Managing the nodulating Rhizobium bacterium is an old, well developed technology. Legume seed are inoculated with Rhizobium in a carrier prior to planting. This has been done long enough in some areas that Rhizobium populations in the soil are so high that seed inoculation no longer results in increased nodulation. Rhizobium strains have been developed that are more efficient nitrogen fixers in the growth chamber, but they have not proven effective in the field, presumably for lack of fitness.

Ectomycorrhizae, common on woody plants, can be cultured on artificial media. It is endomycorrhizal fungi, however, that show such promise on their herbacious crop hosts. They grow only on the living plant, thus complicating their study, increase, and introduction to new plants and areas. Seed or transplants must be treated with mycorrhizal roots or with soil and debris from around the roots of established plants to increase inoculation levels above that from established species. Mycorrhizal fungi need to be established in the host roots some weeks prior to attack to condition protection from a pathogen.

5. Cropping-system and ecosystem-component interactions

Only the tip of iceberg has been touched for interactions research needed in this area. Research to develop still better IPM programs covers some of the need for studying pest interactions. Integrated pest management has become integrated production management or integrated crop management in some states, and these broaden the research considerably. Research of minimum or conservation tillages considers other aspects, as do fertility trials and especially maximum yield research. But I am not aware that any of these takes the holistic agroecosystem approach required to choose among the many interacting cropping systems and ecosystem components to find the positive synergism to recommend to growers.

6. Population biology/genetics of ecosystem components

Pathogen populations have been being subdivided into physiologic races for over 60 years, since the physiologic race concept was advanced by E. C. Stakman. Races of a pathogen population are described in terms of a defined set of differential host varieties. As the genetics of resistance of a given host variety became known, these data were interpreted in terms of Flor's gene-for-gene hypothesis. While the mode of inheritance of resistance in many differentials remains a mystery, this still has given considerable genetic meaning to race identification work. More questions have been raised than answered, however. These relate to all aspects of resistance/virulence theory. The monoclonal antibody technology should hasten progress in this area.

The host cultivar determines the composition of the pathogen population. Most pathogen species, like other microorganisms, are highly variable genetically. With short generation times they can quickly have variants arise to match new resistant cultivars and, recently, fungicides. The cultivar or fungicide then gives the new variant opportunity over its competitors. The desired goal of durable resistance means that the pathogen population can be kept relatively avirulent, or that dilatory resistance will cause the pathogen to develop slowly enough that the crop will mature without significant damage. How to achieve durable or dilatory resistance genetically is far from clear, however, and this is a major need if genes are to be transferred intelligently in a genetic engineering laboratory and managed well in the field.

Few genes in interacting host-pathogen populations have been tracked genetically, and they must be. In England, Wolfe and Barrett made an excellent start with the barley powdery mildew system. Using 3-way mixtures of barley cultivars, each of which carried a different major-effect resistance gene, they found that each cultivar quickly favored its own strain of the haploid pathogen. Even though each mixture component soon became susceptible to and selected for "its own race", the host population remained 2/3 resistant, which is well in excess of the 1/3 - 1/2 resistance needed to protect a diverse plant population. Significantly, the feared "super race" of the pathogen did not develop.

Much more work of this type is indicated in population biology/genetics among all of the biotic components interacting in each agroecosystem.

C. Genetic Engineering

This is extremely promising, as indicated by the number of recently established genetic engineering companies, but in its relative infancy for plants. Plant disease resistance is cited frequently as the area where most pay-off is portended from plant genetic engineering. Unfortunately, as explained above, the genes easiest to transfer are those major-effect genes least welcomed by many breeders. Accepting the field-management technology of (II.B.2.b.) above, however, may make major-effect genes more acceptable to breeders. Also, the promise of genetic engineering may be a major incentive to using that technology. Thus, the field-management technology of (II.B.2.b.) may be one of the fastest ways simultaneously to control certain highly epidemic diseases (e.g., the cereal rusts and mildews) and to reap the benefits from genetic engineering.

The first payoff from genetic engineering in plant disease control may come from manipulating biocontrol agents. For instance, modifying a fungus through mutation or recombinant DNA technology to be both biological antagonist and resistant to a fungicide has enabled the two tactics to be integrated. Then, the fungicide and the antagonist are applied together. The fungicide checks development of the pathogen while the antagonist is becoming established and competitive as a biocontrol agent.

D. Bacteriocides, Fungicides, Nematicides, and Other Chemicals

Work is being done to develop pesticides with more than one site of activity on the molecule, formulations for slow, controlled release, and predictive systems for using pesticides more successfully. However, much more basic research must be done to characterize the range of chemicals that can be translocated in the plant, especially basipetally in the phloem, before target systemic pesticides can be developed that are effective and safe for use.

Most R&D work on agricultural chemicals is done by the private sector. Unfortunately, the current regulatory climate in the USA and the resultant high cost of research, development, and obtaining EPA clearance do not favor adequate activity.

IV. Major Breakthroughs Needed

A. Crop Loss Assessment

Modeling total crop and agroecosystem loss partitioned by cause, including adverse environments, must be nurtured for crops, cropping sequences, different stress sources and environments, and interactions among them, for use both in management and in setting research priorities. The computer-assisted model of Main et al. (1984) is a result of a pilot project sponsored, in part, by the USDA/CSRS National Crop Loss Design Committee. This model should stimulate research to lead to a National Crop Loss Assessment System (NCLAS) as proposed by Dr. John Fulkerson, USDA/CSRS, and the Ad Hoc National Crop Loss Design Committee. The NCLAS should be incorporated eventually as an integral part of the National Plant Health System proposed in Section IV, Institutional Arrangements.

New funds needed: $3,000,000 per year, increasing gradually to $8 million as crop loss assessment matures and becomes institutionalized into NCLAS.

B. Cropping-System and Agroecosystem-Component Interactions

While this category was subdivided into six parts for presentation in Sections II and III, conceptually it must be researched as a unit even though individual scientists may concentrate on only a portion of a multidisciplinary experiment. Initially, pilot projects should be begun at 1-2 experimental stations in each of the four regions of the USA, i.e., Northeastern, Southern, North Central, and Western Regions. Each station should have sufficient land, resources, and administrative and disciplinary personnel committed to the project that it can set up and conduct long term experiments. These should include plots of key commodities of the region, be replicated and randomized, and be large enough that additional treatments can be added as dictated by experience without changing the original experiments seriously. Major crops and several different cropping sequences and husbandry practices must be included. Different modes of field management of resistance genes will be tested, as will different pesticide alternatives. A tremendous amount of monitoring of pest populations will be necessary aided by pheromone traps, pathogen-specific media, isogenic trap crops, monoclonal antibody technology, etc. This computer-assisted study will develop and test interaction models and generate SYAE recommendations to be tested elsewhere in the regions.

This collective experiment will seem expensive. Indeed, the necessary monitoring of pest populations required for genetic interpretations of the interactions will not come cheap. But this will be economical relative to return on investment, and be necessary to detect those synergistic interactions most likely to provide the next breakthroughs in our already well developed agricultural system.

A country that can assemble a team and put a man on the moon can do no less than assemble a team as proposed here to do this forced pace, holistic, agricultural research. Such will be necessary if there is to be hope of feeding the world's 6.25 billion people expected by 2000, much less the 10 billion predicted by 2050.

New funds needed: $5,000,000 per station for a total of ca. $40,000,000.

C. Genetic Engineering

Many of our resistance/virulence management theories are being formed from a very limited knowledge base. Fortunately, we do not have to know how a gene works in order to use it conventionally. The genetic control of wheat resistance to stripe rust was first shown in 1905, and scores of rust resistance genes have been used since without our knowing their mechanism of action. If we knew how they do what they do, however, we probably could manage them more successfully. This should be possible with contemporary technology.

Probably the key products of resistance genes are antigens. It is probable, therefore, that the highly specific new monoclonal antibody technology can provide the necessary probes to identify and quantify enough of the key gene products to permit the parent gene(s) to be identified and isolated. This is an important step in understanding the resistance/virulence genes well enough to bring genetic engineering techniques to bear on the problem of transferring functional resistance gene systems into agriculturally important crops.

Corn is the best known genetically of all the higher plants. Yet the ca. 200 genes that are well known in corn are not those used in breeding cultivars for commercial production; they are more likely to be the deleterious chlorophyll or other mutants. Possibly the most valuable and easily identifiable (by the pathogen) major-effect genes are those that condition disease resistance. We must get on with developing the recombinant DNA technology to transfer these genes successfully, and simultaneously test the paradigm of diversity so this valuable resource -- the major-effect resistance genes -- can be managed well, as in (II.B.2.b.) above. Developing this technology should move us further toward our goal of transferring groups of minor-effect genes.

Monoclonal antibody technology may also bring clarification of major- versus minor-effect genes, the one resistance gene-for-one virulence gene relationship, and the probable durability of each type. It could facilitate their being tracked by the population geneticist and lead to more enlightened gene management.

Possibly, also, the gene product that effects resistance can be synthesized biologically through genetic engineering, giving us a valuable new disease management technology.

New funds needed: $10,000,000 per year.

D. Bactericides, Fungicides, Nematicides, and Other Chemicals

Additional work is needed at all levels on pesticides, but especially to complete development of systemic pesticides with two sites of activity on the molecule to extend the pesticide's useful life. Research pesticide-host-pathogen interactions and identify secondary compounds and other novel products formed. A great need is for systemic chemicals to be applied to seed or foliage, be absorbed, translocate basipetally to the roots, and move into the rhizosphere to inhibit a target pathogen or stimulate beneficial antagonists. A related need is for systemic pesticides that can be injected or absorbed into a plant's vascular system, translocate, and kill an infectious agent. Basic physiological research to characterize acropetal- and basipetal-translocatable-type compounds is needed. Then, possibly pesticides could be synthesized on those models.

For nematicides, chemical confusants may be possible that will interfere with feeding, mating, hatching, or dormancy, or promote them out of season or sequence. For endoparasitic nematodes, of which the pinewood nematode may be an extreme example, systemic chemotherapeutants are indicated. This work probably will be done and funded by private industry.

Naturally occurring chemicals should be sought that stimulate the plant's defense mechanisms or that enhance activity by biocontrol agents. Ultra low volume delivery systems must be sought for these as for regular pesticides that are active in very low dosages.

Additional funding needed: $5 million per year is needed, half of it to support the type of short-lived, sensitive radioactive ^{11}C studies required.

V. Institutional Arrangements

Currently, research in most areas discussed is conducted by the federal government and by the state agricultural experiment stations in the Land-Grant University System, with coordination nationally through the USDA/CSRS and ESCOP. Research and development on pesticides are conducted primarily by private industry and breeding of new cultivars is being done increasingly by the private sector. Much of the testing and recommending of new pesticides, cultivars, and cultural systems is by the Cooperative Extension Service. Private industry is very active in genetic engineering. Generally, scientists in each agency and sector work well together with testing back and forth, and with memoranda of understanding and patenting and licensing agreements, as indicated.

But for the future, new institutional arrangements are needed to give plants their due. In Section (I.A.) I suggested that plant pathogens should be managed as a part of IPM, which leads naturally to the still broader concept of plant health. This broader concept is the umbrella that could and should enfold all the disciplines that relate to sources of biotic and abiotic stresses, not just plant pathogens. This is true because the common thread that ties all of the plant health-related sciences and activity together is man's obligate dependence on the green plant -- nature's primary photosynthetic factory -- and his imperative to keep it healthy and productive. Accepting the green plant, and for our purposes its health, as our central focus is more of a paradigmatical than an institutional problem. Recognizing the central importance to mankind of the healthy productive green plant should lead to the establishment of a National Plant Health System (NPHS) to give the health of the green plant the importance and attention it deserves.

I visualize, and have proposed, that the NPHS evolve from present plant-health-related disciplines in the Land-Grant University System with its federal linkages. I think it should be coordinated by an Assistant Secretary of Agriculture for Plant Health and have standing equivalent to the prestigious National Institutes of Health.

I see the NPHS as operating in four modes: Research, Delivery, Education, and Reporting. Research will place and keep plant health on the cutting edge of science, and develop sustained yield agro- (or horticultural or silvicultural) ecosystems that Delivery can recommend as an integrated package to growers. Delivery will increasingly be by a new professional "Doctor of Plant Health" that will parallel the Doctor of Veterinary Medicine. These general practitioners will be backstopped by research and, especially, extension specialists in the several plant health-related disciplines. Education will produce paraprofessionals and the new professional Doctor of Plant Health. And for Reporting, the new corps of paraprofessionals, observer-reporters similar to entomology's scouts, will cover the crop and report crop conditions and pest incidence for management, new pests for USDA/APHIS, and data for USDA/SRS/CRB to make crop yield and loss estimates, partitioned by cause. Of course, many of the Delivery and Reporting functions will be done by the "early adapter" growers themselves, who will be better prepared to do so by Extension.

I must emphasize again that the focus of the proposed NPHS will be a very positive one. It must focus on the green plant and keeping it and its ecosystem healthy and productive. It is this central focus, I feel, that causes the plant health concept to differ from other advanced, imaginative concepts like ICM (Integrated Crop Management), MRSM (Multiple Resource Systems Management), and other gestating proposals. Recognizing the central importance to man of the healthy, productive, green plant and seeing to its needs and the needs of its ecosystem enable the legitimate causes of other helpful acronyms to fall into place.

Plants will inevitably become more valuable as the population of humankind continues to increase. It is the economic necessity of overseeing the health of the nation's crops that, eventually, will cause agriculturists, scientists, and politicians to see the imperative of a National Plant Health System. Meantime, the research proposed is still needed, and the Cooperative Extension Service can continue as the main interface between researchers and growers.

VI. Likely Timing of Commercial Introduction (assuming that the past trend in research support will continue into the future)

A. Develop the Science of Crop Loss Assessment

Here, I interpret continuation of past research support to include the pilot projects funded by USDA/CSRS. This support has already produced the report of Main et al. (1984). This level of support and activity will stimulate a great deal of additional interest and significant commercial impact by 2000. With the funding level proposed, however, a National Crop Loss Assessment System could be operational by 1990.

B. Cropping-System and Agroecosystem-Component Interactions Research

With the present level of funding, no significant commercial impact is foreseen from interactions research. Obviously, if this area is of any importance, the present level of non-funding cannot continue.

In fairness, considerable resources are currently allocated to breeding resistant cultivars by both the public and private sectors. This is true to a lesser degree for plant fungicide, nematicide, and bacteriocide development and still less for biocontrol agents. This work should continue. But the type of holistic research proposed is necessary to put it all together. With the funding proposed, I predict significant impact by 1995.

C. Genetic Engineering

The potential of plant genetic engineering is now generally realized and relatively well funded; much of it with venture capital. Thus, present and anticipated research at the current level of funding may have products ready for commercial introduction into grower's fields by 2000. The limiting factor here may be the time required to test the cultivar or biocontrol agent prior to its release for commercial use and ensure that adequate fitness has been incorporated along with the targeted major-effect gene. Organisms genetically engineered for use in biological synthesis and in other ways where the substrate and environment are controlled artificially and competitive fitness is of little concern should impact commercial agriculture by 1990. Of course, all of this should be preceded by an explosion of basic knowledge of gene action, gene control of developmental biology, host-pathogen interaction, etc., and who can predict where that will lead -- or when?

D. Bacteriocides, Fungicides, Nematicides, and Other Chemicals

The current level of support probably is not the factor limiting R&D of this group of pesticides and other chemicals. The limiting factor is the regulatory climate that makes pesticide research, development, and testing to get government clearance so expensive and problematical as to discourage this use of venture capital. Meantime, however, much basic research should be being done by the public sector on chemicals that translocate in plants, on factors that control that process, and on other naturally occurring products that affect the host-pathogen interaction.

I anticipate that definitive work on crop loss will establish that plant diseases are of such economic importance, and therefore the potential market for pesticides so great, that additional work will be done on pesticide R&D, most of it by the commercial sector.

I am optimistic for impact on commercial agriculture by 1990.

VII. Potential Primary Impacts

The potential impact, if the technology and institutional arrangements proposed herein are accepted by the agricultural community and the nation, should be sustained yield agricultural (agronomic, horticultural, and silvicultural) ecosystems focusing on the increasingly valuable green plant and overseen by a National Plant Health System (NPHS). The proposed National Crop Loss Assessment System would be incorporated into the NPHS, as would most of the present plant health-related research, teaching, and extension activities of the USDA and the Land-Grant University System. This finally would give plants the attention due them as the primary photosynthetic factory on which man is totally dependent. It is not a question of whether plants will receive this attention, but when. The USA should get on with the NPHS for its own good and as a model for the rest of the world. The sooner it happens, the more secure world agriculture -- and therefore the world -- will be.

Short of the NPHS, the technology proposed can still have tremendous impact. The science of crop loss assessment should be developed and made operational both for use in setting research priorities and in making management decisions. Possibly the most important spin-off of crop loss assessment is in demonstrating convincingly the magnitude of losses from plant diseases (and other sources of plant stress) and the stimulus this will be to public and private funding agencies and industry to support imaginative research on plant diseases and develop control measures. If so, this will be following the pattern set in Western Europe.

Researching cropping-system and ecosystem-component interactions is the surest way to develop high level, sustained yield agroecosystems. In them, the protection a cultivar enjoys should be a product not just of the individual plant, as commonly in the past, but of the entire plant population, agroecosystem, and cultural system. The USA has a highly developed agriculture. Breakthroughs to further improve such a highly developed agriculture are most likely to come from research of interacting cultural practices and agroecosystem components to identify those that are positively synergistic. Components to be studied include each new generation of agronomically superior, disease resistant cultivars, biocontrol agents, pesticides, etc. that are portended from advances in genetic engineering. The field management technology for disease resistance genes proposed herein may be the surest way to quickly stabilize USA agriculture against certain highly epidemic pathogens and to reap maximum benefit from plant genetic engineering.

VIII. General References

Andrews, John H. 1983. Future strategies for integrated control. p. 431-440. In: Thor Kommedahl and Paul H. Williams, Eds. Challenging Problems in Plant Health. The American Phytopathological Society, St. Paul, MN 55121. 538 pp.

Baker, Kenneth F. 1983. The future of biological and cultural control of plant disease. Ibid, p. 422-430.

Brewbaker, James L. 1983. Breeding for disease resistance. Ibid, p. 441-449.

Browning, J. Artie. 1983. Goal for plant health in the age of plants: A National Plant Health System. Ibid, p. 45-57.

Buddenhagen, Ivan W. 1983. Agroecosystems, disease resistance, and crop improvement. Ibid, p. 450-460.

Cook, R. J., and K. F. Baker. 1983. The Nature and Practice of Biological Control of Plant Pathogens. The American Phytopathological Society, St. Paul, MN. 55121. 538 pp.

Delp, Charles J. 1983. Changing emphasis in disease management. p. 416-421. In: Thor Kommedahl and Paul H. Williams, Eds. Challenging Problems in Plant Health. The American Phytopathological Society, St. Paul, MN 55121. 538 pp.

Fry, William E., and B. G. Tweedy. 1983. Introduction to disease control. Ibid, p. 401-404.

Kommedahl, Thor, and Paul H. Williams, Eds. 1983. Challenging Problems in Plant Health. The American Phytopathology Society, St. Paul, MN 55121. 538 pp.

MacKenzie, D. R. 1983. Toward the management of crop losses. Ibid, p. 82-92.

Main, C. E., S. M. Nusser, and A. W. Bragg. 1984. 1983 crop losses in North Carolina due to plant diseases and nematodes. Department of Plant Pathology Special Publication No. 3, North Carolina State University, Raleigh, NC. 146 pp.

Teng, P. S., and R. J. Oshima. 1983. Identification and assessment of losses. p. 69-81. In: Thor Kommedahl and Paul H. Williams, Eds. Challenging Problems in Plant Health. The American Phytopathological Society, St. Paul, MN 55121. 538 pp.

Tweedy, B. G. 1983. The future of chemicals for controlling plant diseases. Ibid, p. 405-415.

Wolfe, M. S. 1983. Genetic strategies and their value in disease control. Ibid, p. 461-473.

Background Paper No. 13

Management of Insects and Mites

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MANAGEMENT OF INSECTS AND MITES

Summary

Insects and mites are mankind's greatest competition for food and fiber. Although less than 1 percent of all insect and mite species are considered agricultural pests, those pests cause average annual losses to agricultural production of 5-15 percent, despite the expenditure of millions of dollars each year for agricultural pest control. Thus, protecting crops from such losses will continue to be an important component of agricultural production.

Research on this problem is being conducted in the broad areas of (1) chemical controls for insects and mites, (2) genetic manipulation of plants and insects and their natural enemies, and (3) information processing.

Because they are highly effective, economical, and fast-acting, chemical insecticides and acaricides (for mites) are widely used for reducing insect and mite populations to subeconomic levels. Advances in insect physiology, toxicology, and analytical chemistry are leading to the discovery of new compounds that disrupt the normal growth and development processes of insects. Compounds with juvenile hormone activity that prevent an insect from molting to the adult stage, those with antijuvenile hormone activity that cause insects to molt prematurely to the adult stage, and those that interfere with the normal synthesis and deposition of exoskeleton all hold promise for the future. Similarly, advances in the chemistry of natural products and the study of plant defenses against insects and mites are leading to the identification of naturally occurring, insecticidal and acaricidal compounds with novel modes of action. Many such compounds are likely to be suitable for large-scale production via fermentation processes with genetically engineered microorganisms.

With existing application technology only 25-30 percent of a pesticide is actually deposited on plant surfaces and less than 1 percent actually reaches the plant. In addition to being wasteful, this situation greatly exacerbates undesirable effects to the environment. One factor is the incorrect mixing and calibration by pesticide applicators. Efforts are thus being made to design equipment that injects pesticides at the proper rate directly into the lines carrying water to the nozzle, eliminating the need for tank mixing. Other research will ensure more uniform droplet size, will control spray drift, and will improve adherence of the spray to the plant.

Advances in genetic engineering greatly increase the likelihood of new classes of insecticides and acaricides. Insect pathogens, including bacteria, fungi, protozoa, and viruses, are likely candidates for genetic enginerring to enhance their utility as microbial insecticides. The pathogenic bacterium *B. thuringiensis* is already commercially available and widely used to control caterpillars on certain crops. Genetic enginerring holds great promise for expanding the spectrum of pests controlled by *B. thuringiensis*.

Crop varieties resistant to insect pests have been used to manage insects with success in a number of important crops. Use of genetic engineering to transfer genes from resistant wild plants to crop cultivars holds great potential for insect and mite management, but requires very specific knowledge of the biochemical bases of the resistance to be transferred. In most cases, the requisite knowledge is not yet available.

Improvements in the design and availability of computer hardware and software will produce tremendous changes in insect and mite management at the research, extension, and farm levels. To contribute to crop profitability, insect and mite management entails the processing of tremendous amounts of information on the condition and the phenological stage of the crop, the status of insects and mites and their enemies in the crop, incidences of plant diseases and weeds and measures used in their control, weather conditions, crop production inputs, and insect and mite management options. Computers at the farm level, with access to centralized data bases, will allow farm operators to design and implement pest management strategies for their farms. Some software systems are already in place and are continually being improved. In general, however, improvements in data bases are awaiting advances in knowlegdge about pest dynamics and crop pest interactions.

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INTRODUCTION

Insects and mites are man's greatest competitors for food and fiber. Although less than 1% of all insect and mite species are considered agricultural pests, those pests cause average annual losses to agricultural production ranging from 5 to 15% despite the expenditure of hundreds of millions of dollars each year for agricultural pest control (Bowers, 1983; Davidson and Lyon 1979; Pimentel et al. 1978). For this reason, the protection of agricultural crops from losses due to insects and mites will continue to be an important component of agricultural production for the foreseeable future and beyond.

The history of insect and mite control in agricultural crops, especially during this century, is one of accelerating technological innovation, if not revolution. Early in this century, controls were largely cultural involving the selection of planting dates, tillage, sanitation, crop rotation and other practices to reduce the size of the pest populations attacking the crop and to take advantage of the naturally-occurring biological control resulting from the actions of parasites, predators and pathogens of the pest species. The insecticides available were inorganic compounds (e.g. lead arsenate, calcium arsenate) and botanicals (plant derived compounds - e.g. rotenone, pyrethrins). They were of marginal effectiveness as judged by modern standards.

Beginning in the 1940's the introduction and widespread use of synthetic organic insecticides led to the almost complete reliance on insecticides and acaricides for insect and mite control. However, by the mid 1960's and 70's problems with this approach became apparent. Those problems include, but are not limited to: 1) selection of insecticide resistant pest populations; 2) induced outbreaks of secondary pest species following destruction of their natural enemies; 3) resurgence of target pest populations, following insecticide treatment, to higher levels than were present before treatment; 4) undesirable effects on non-target organisms including parasites and predators, honey bees, fish, birds and other wildlife, man and domestic animals and the crop plant; 5) direct hazards associated with the application of pesticides; and 6) hazardous residues of pesticides on the harvested crop (Smith 1970). The widespread recognition of these problems led to the conceptualization and general acceptance of Integrated Pest Management (IPM) as the operational paradigm for protecting crops from losses to insect, mite and other pests (Glass 1975; Huffaker 1980; Metcalf and Luckman 1975; Rabb and Guthrie 1970; Sill 1982).

DEFINITION AND SCOPE

The IPM paradigm places crop protection within the larger context of crop production, the objective of which is to optimize production and not to eliminate pests. Within the broader framework of this paradigm, insect and mite pest management has been defined by an FAO Panel of Experts as a:

> "system that in the context of the associated environment and the population dynamics of the pest species, utilizes all suitable techniques and methods in as compatible a manner as possible and maintains the pest populations at levels below those causing economic injury."

It is within the broad context of IPM that developments in the technology of insect and mite control must be viewed. For the purposes of this paper the technology of insect and mite control is considered to include not only the direct control tactics used to suppress insect and mite pest populations to sub-economic levels (e.g. insect and mite resistant crop cultivars, cultural controls, biological control, autocidal control, insecticides and acaricides, insect growth regulators and insect behavior modifying chemicals (see Glass

1975; Nordlund et al. 1981; Whitehead and Bowers 1983), but the strategies for the selective deployment of these tactics. Also included in this technology is biological and ecological information necessary to develop and utilize the control tactics in IPM, and the techniques, procedures, and systems for information processing and delivery.

MAJOR LINES OF RESEARCH

Basic and applied research of relevance to the control of insects and mites of agricultural importance is being conducted at the State Agricultural Experiment Stations, public and private universities, within the USDA/ARS and in private industry. The fruits of much of this research will gradually be incorporated into crop production and pest management systems at all levels. The benefits of this research should not be overlooked merely because they lead to gradual and subtle, rather than major and rapid, changes in the approach of American agriculture to insect and mite control.

Research in the broad areas of 1) chemical controls for insects and mites, 2) genetic manipulation of plants as well as insects and their natural enemies, and 3) information processing offer, perhaps, the greatest potential for the development of major new technologies. The development and successful deployment of these technologies will be dependent upon continued advances in our understanding of the target biological and ecological systems.

Chemical Controls

The reliance and over-reliance on insecticides and acaricides for pest control in the past have created many of the problems associated with their use. Other problems are related to the nature of the chemicals used (e.g. persistance and biomaginification of some chlorinated hydrocarbons) and the prevailing application technology. The IPM concept seeks to minimize the problems associated with insecticide use and maximize the advantages (Metcalf 1975). Because they are highly effective, economical and fast-acting, insecticides and acaricides provide a unique tool for restoring damaging insect and mite populations to subeconomic levels. For this reason, they will remain an important tool for use in the management of insect and mite pests of agricultural crops. Major lines of research underway will make available new types of compounds for use as insecticides and acaricides, and will lead to significant changes in the way these pesticides are used in many crops.

New Kinds of Insecticides and Acaricides - Research leading directly to the discovery and development of new insecticides and acaricides is conducted primarily by the agrochemical industry. However, much background and support research is being conducted by scientists in public and private universities, government laboratories and state agricultural experiment stations. Most of the insecticides currently in use were discovered using the conventional strategy for pesticide synthesis. This strategy consists of identification of a pesticidally active molecule followed by the synthesis and bioassay of structural analogues of the original active molecule which were selected for synthesis on the basis of the relationship between molecular structure and pesticidal activity (Menn 1983).

Virtually all of the major insecticides in widespread commercial use on agricultural crops are acute neurotoxins and represent a rather limited range of chemical classes: chlorinated hydrocarbons, organophosphates, carbamates and pyrethroids. The pyrethroids represent the class of insecticides currently receiving the greatest development efforts by industry. Although these compounds are more expensive, they are effective at much lower rates than are most compounds in the other classes.

Advances in our knowledge of insect physiology, toxicology and analytical chemistry are leading to the discovery of new compounds with novel modes of action that disrupt the normal growth processes of insects. The juvenile hormone analogues, for example, prevent the insect from molting to the adult stage. Unfortunately, because it is the larval stages which are most damaging to crops, these compounds appear to have limited use in agricultural crop protection. They are, however, highly useful against insects, such as fleas, biting flies, etc. which are pests in the adult stage. Compounds with anti-juvenile hormone activity, which cause insects to molt prematurely to adults have been discovered (Bowers et al. 1976). These anti-juvenile hormones offer promise for use in the management of agricultural insect pests. The relatively recent discovery of a group of compounds which disrupt the molting process of insects by interfering with the synthesis and deposition of chitin, a principal component of the exoskeleton of insects, also holds promise for the future (Verloop and Ferrell 1977).

Similarly, advances in natural products chemistry and the study of plant defenses against insects are leading to the identification of numerous, naturally-occurring, insecticidal and acaricidal compounds with novel modes of action (Rosenthal and Janzen 1979; Whitehead and Bowers 1983). To date, biologically active natural products have been looked to by the agrochemical industry as leads for the chemical synthesis of structurally related compounds with improved biological and physical properties and which are amenable to large scale chemical synthesis (Pryce 1983). This latter requirement, in many cases, will become of lesser importance with advances in genetic engineering, since even complex molecules could be produced on a large scale using fermentation processes with genetically engineered microorganisms.

Insecticides and acaricides with novel modes of action and differing degrees of specificity will provide new options for agricultural pest managers. They will also facilitate the management of insecticide resistance in key pest problems. Their effective use, however, may well require the development of novel approaches to utilizing chemical control agents for insect and mite management.

Insecticide/Acaricide Application Technology - With existing application technology only 25 to 50 percent of the pesticide is actually deposited on the plant surfaces and less than one percent actually reaches the pest species (Metcalf 1980). In addition to being wasteful, this situation greatly exacerbates undesirable nontarget effects of insecticide and acaricide use. As pesticides become increasingly expensive and the need to eliminate off-target effects becomes more pressing, there will be major changes in the ways in which insecticides and acaricides are applied to crops. The development of new pesticide application technology is well underway and will be widely available commercially in the foreseeable future. The rate at which it becomes adopted will depend upon the prevailing economic and regulatory incentives.

One major source of inefficient and ineffective pesticide use is improper mixing of pesticides and calibration of pesticide application equipment. One recent survey found that two out of three pesticide applicators make significant and costly application errors due to incorrect mixing and calibration (Reichenberger, 1980). To obviate these problems, efforts are underway to design equipment which injects pesticides at the proper rate directly into lines carrying the water to the nozzle, thus eliminating the need for tank mixing (Reichard and Ladd 1983).

Even when application equipment is properly calibrated, the plant being sprayed receives and retains only a small portion of the pesticide sprayed. Several approaches have been taken to improve droplet impingement onto the plant target. Research on controlled droplet application (CDA) technology has produced several types of droplet generators for ultra-low volume (ULV) (<0.5 gal/A) field application of pesticide. The more uniform droplet size produced by these generators provides better control of plant canopy penetration and the low volumes required reduces application costs (Clower et al. 1982). One problem with existing CDA and ULV technology is that the small droplet sizes result in enhanced spray drift. Progress is being made in overcoming this problem by imparting a slight negative charge to the droplets so that they are attracted to the plant, which carries a slight positive charge (Law 1978; Law and Lane 1980). Electrostatic deposition techniques produce a more even distribution of the pesticide on the plant than conventional techniques (Law 1981; Lane and Law 1982). Because of the better plant coverage provided by electrostatic spraying, effective insect control can be achieved using significantly reduced quantities of insecticide (Manley 1982). The current slow commercial development of electrostatic sprayers is due, in part, to the special pesticide formulations required. While these are being developed, a practicable method of charging conventional water-base spray formulations is also under investigation (Marchant and Green 1982).

Another approach to better targeting pesticide sprays is the automatic intermittent sprayer developed for horticultural crops (Ladd et al. 1978). Using pulse-modulated IR or photoelectric sensors to detect plants and trigger insecticide release only on the plant, an intermittent sprayer uses 25 to 50% less insecticide than a conventional sprayer on cole crops and peppers (Ladd et al. 1978; Ladd and Reichard 1980). When used in conjunction with an action threshold, an intermittent sprayer resulted in even greater savings in pesticides than either a conventional sprayer used with an action threshold or the intermittent sprayer used weekly (Ladd et al. 1981).

Advances in insecticide formulation technology such as microencapsulation and slow release technology are being made which hold great promise for enhancing efficacy, tailoring residual activity and minimizing nontarget effects of insecticides.

The potential benefits in terms of reduced nontarget effects and savings to the farmer and the public resulting from improved formulation and application technology are great, especially when that technology is coupled with the development of improved pest monitoring techniques and action thresholds.

Genetic Engineering

The technology of genetic engineering, which allows the movement of specific genes and gene sequences between organisms, is discussed elsewhere (Fraley pages). This technology holds great promise for numerous innovations in arthropod management. Since this area is so new, the full extent of the potential applications of genetic engineering, undoubtedly, has not, as yet, been fully recognized. Some of the most obvious and, perhaps more immediate, applications are presented below.

Bacterial Production of Natural Products for Use As Insecticides and Acaricides - The ability to introduce plant genes into bacteria opens the way for the genetic engineering of bacteria to produce plant natural products with insecticidal, acaricidal or insect growth regulator activity. This would enable the large-scale production and use of natural products as pesticides even though the specific molecules might be highly complex and too costly to produce via standard chemical synthesis. This technology greatly expands the likelihood that new classes of insecticides, acaricides and insect growth regulators with novel modes of action will be available for use in insect and mite management systems.

Genetic Engineering of Insect Pathogens - Insect pathogens, including bacteria, fungi, protozoa and viruses, are important mortality agents in the population dynamics of many insect and mite pests. Pest management programs for some crops seek to maximize the impact of naturally-occurring pathogens on insect and mite pest populations. However, for a variety of reasons including (but not limited to) problems with quality control, pathogen selectivity, environmental instability, requirements for mass production and an uncertain regulatory environment, relatively few insect pathogens have been developed for commercial use. The pathogenic bacteria Bacillus thuringiensis is commercially available and widely used to control caterpillars on certain crops. Another bacteria, B. popilliae has been widely used to control the Japanese beetle. Several fungi (Roberts et al. 1981) and insect viruses (Falcon 1976; Tinsley 1979) are being investigated for potential commercial use as insect control agents. In most situations these pathogens will be used in pest management as microbial pesticides. Many of the biological limitations of the pathogenic organisms which have impeded their development as microbial pesticides can potentially be eliminated through genetic engineering. Insect pathogens, for example, might be genetically engineered to 1) alter their host range; 2) improve their epidemiological characteristics; 3) improve their environmental stability; 4) develop more potent strains; 5) eliminate undesirable characteristics from existing strains; 6) improve their mass production (Levin 1979). Genetic engineering efforts to improve insect pathogens are still in the earliest stages of development but will undoubtedly produce advances in the use of insect pathogens for pest management.

Genetic Engineering of Plants - Crop varieties resistant to insect pests have been used in the management of insect pests with great success in a number of important crops (Painter 1951; Harris 1980; Maxwell and Jennings 1980). Ideally, insect and mite resistant crop varieties should be important components of the pest management programs on most crops, since their use seeks to take advantage of the plants' natural defenses against and tolerance of pest attack. In practice, the use of insect and mite resistant varieties has the advantages of: 1) involving little or no added costs to the farmer; 2) requiring little or no added pest control skills on the part of the farmer; 3) generally being compatible with other management tactics; and 4) involving no toxicity, residue or environmental hazards.

The basis for developing insect and mite resistant varieties stems from our ability to identify those plants which, because of their genetic make-up, are less attacked and less damaged by one or more pest species than other plants under comparable levels of pest attack. However, it is contingent upon our ability to transfer the genes conferring the pest resistance from the resistant plant to crop varieties having all of the requisite agronomic or horticultural attributes. Traditionally, this has been done using standard plant breeding techniques and we have been restricted in our sources of resistance genes to plants of the same species as the crop being bred or to closely related species which will cross readily with the crop plant. The technology of plant genetic engineering promises to free us from this limitation since genes from virtually any source can be introduced into and expressed in plants. Thus, genetic engineering offers the potential of a virtually unlimited germplasm base (in theory any plant species) from which to select genes conferring insect or mite resistance for incorporation into a crop variety.

Utilization of genetic engineering to this end requires very specific knowledge of the biochemical basis of the trait to be transferred. Although considerable research is being conducted on the biochemical mechanisms of plant resistance to arthropods (e.g. Rosenthal and Janzen 1979; Hedin 1983), more research and more detailed research of this type is needed before the potential for genetic engineering in the area of plant resistance to insects and mites can be realized. As discussed elsewhere (Fraley), additional advances in the area of plant biotechnology are also needed. It should be emphasized that, even when these advances have been made, at least for the foreseeable future, genetic engineering will not eliminate the role of traditional plant breeding in the development of insect and mite resistant crop varieties. Rather, its primary contribution will be to streamline the search for resistance genes and make available to the plant breeder many more sources of pest resistance genes.

Information Processing

Improvements in the design and availability of computer hardware and software will produce tremendous changes in insect and mite management at all levels (research, extension, pest management presonnel and farmer). To be efficiently implemented, as measured by its contribution to crop profitability, insect and mite management requires the processing of tremendous quantities of information including; a) condition and phenological stage of the crop; b) status of the various insect and mite pests and their natural enemies present in the crop; c) production inputs into the crop; d) incidence of plant diseases and weed pests and the measures used in their control; e) weather conditions and f) insect and mite management options. Further, this information must be updated and reviewed at regular intervals. Computers can help tremendously in the effective and efficient processing of this information as well as in the design, direction and analysis of pest management related research.

It has been predicted that the number of farms using programmable devices will approach 75% by 1990 (Welch 1984). The availability at the farm level of microcomputers equipped with appropriate software and having access to larger centralized data bases will greatly speed the transfer of information and facilitate pest management decision making. The advantages, simply in terms of information storage and retrival, will be tremendous. The ready storage of and access to current and historical information on pest biology, incidence and abundance, pesticide use, cropping histories, weather, etc. at the regional, farm and even field level will facilitate the selection of the appropriate management unit and the design and implementation of pest management, strategies for that unit.

Centralized, computer-based, data management systems for crop, pest, and environmental monitoring information have been developed and are being evaluated for use on a regional scale by a USDA/APHIS regional program. Such systems will provide rapid analysis, summarization and access to general crop summaries, observer reports, pesticide and field management information, reports of new or unknown pests, general pest survey information and specified field locations with pest severities.

Other software systems designed to directly facilitate the implementation of pest management programs are in use and are continually being improved. The Prediction Extension Timing Estimator (PETE) model (Wetch et al. 1978) is a generalized model for the prediction of arthropod phenological events. It uses as the principal driving variable for arthropod development heat summations of day degrees. PETE is sufficiently flexible to be used for management in many agricultural and nonagricultural systems. It is most widely used for scheduling scouting and the application of control tactics and as a part of the broader biological monitoring scheduling system (BIOSHED) developed in Michigan by Gage et al. (1982) for a large number of pests on a wide variety of crops (Croft and Knight 1983).

Experiences with these and other software systems has demonstrated their great value and identified areas where improvements are needed. It has also pointed out that the data base, from which biological models are developed is limited. Since all biological models are only as good as the biological information upon which they are based, the continued development and improvement of such models for use in IPM is contingent upon continued high quality research on the appropriate aspects of plant and pest biology and ecology.

The advantages, in terms of improved efficiency and accuracy with which pest management decisions can be made and implemented, provided by the computer software already available are tremendous. There is a great deal of effort currently being devoted to the development of new and the improvement of existing software. This, in conjunction with the rapid advances being made in computer hardware, provides a powerful force which will lead to dramatic changes in the implementation of IPM and to increases in the level of sophistication of IPM where such increases are desirable.

MAJOR BREAKTHROUGHS NEEDED

The degree to which the technologies discussed above and, indeed, any technology, contribute in a positive way to agriculture will in large part be determined by our understanding of the production, biological and ecological systems into which those technologies will be introduced. The introduction of a poorly understood technology or the introduction of a well understood technology into a poorly understood system commonly results in a disproportionate number of unanticipated and often undesirable side effects. The only way to maximize the benefits to agriculture of developing technologies and to minimize their undesirable side-effects is to ensure that the technologies themselves are well understood at the time of their introduction. Perhaps of even greater overriding importance is to ensure that the agricultural, biological and ecological systems affected by agricultural technology are well understood. As much as we presently know about these systems, our level of understanding is woefully inadequate.

The effective utilization of insect and mite management related technology within IPM requires we accept the agroecosystem as the basic unit of IPM related research. The continued development of IPM related technology requires that we greatly enhance our understanding of plant and insect developmental and physiological processes, as well as the nature of the stressful interactions between insects and plants which result in crop losses.

For the technologies discussed above, it is difficult to identify specific major breakthroughs that are needed to advance the technology. However, general areas in which the accumulation of further knowledge will very likely lead to technological advances can be identified.

Chemical Control

Further advances in our understanding of insect physiology, toxicology and plant chemical defenses against insects are needed to facilitate the discovery of new insecticides and acaricides with novel modes of action and which will provide new alternatives for chemical controls used in pest management. In addition, an understanding must be developed of the processes involved in the rapid breakdown of soil applied insecticides by microorganism so that the problem, which is rapidly increasing in severity, can be alleviated. Improvements in our understanding of the ecology, behavior, and population dynamics of pest arthropods and of the responses of crop plants to arthropod attack are needed to take full advantage of developing chemical control technologies and to effect significant reductions in the frequency and rates of insecticide applications to minimize off-target effects and reduce costs.

Genetic Engineering

The principal breakthrough needed for the application of genetic engineering to insect and mite management is the identification, at the molecular level, of useful genes in both plants, insects and insect pathogens. This will require a great deal of basic research on the biochemistry of insect resistance in plants or pathogenesis of insect pathogens. In many cases this will have to be reinforced by a solid understanding of insect population genetics if effective strategies for the utilization of genetically engineered plants and insect pathogens are to be developed. In addition, the appropriate biotechnology (Fraley) required to achieve specific objectives must be developed.

Information Processing

Information processing and computer technology of relevance to IPM has far out-paced the biological data base. Thus, advances are needed in our knowledge of pest dynamics and crop pest interactions so that additional pest phenological models, population growth models and action threshold models can be developed for use in IPM. Biological and environmental monitoring networks having the appropriate degree of resolution for the full range of IPM applications must be established. In IPM, pest, crop and management-decision algorithms are available for use on relatively few crops and pests. There is a great need for the continued refinement of many existing IPM algorithms and the development of new algorithms. Only when this occurs in association with an adequate biological data base will the full impact of information technology be felt in the area of IPM.

INSTITUTIONAL ARRANGEMENTS

As is true for most agricultural research, research activities in each of the areas discussed above are underway in or supported by the Federal Government, state agricultural experiment stations, public and private universities and private industry. University and state agricultural experiment station research is supported by Federal funds in the form of Hatch funds, competitive research grants and cooperative agreements, and by private industry through grants and contracts. Industrial funding and much of the Federal funding is focused on the solution of specific problems. Sources of funding for basic research in support of agriculture are much more limited.

Industry conducts much of its own research in-house, but utilizes, extensively, information generated by university, agricultural experiment station and federal scientists. Especially in the areas of chemical controls, agricultural experiment station and extension scientists assist industry in the final stages of product testing.

LIKELY TIMING OF COMMERCIAL INTRODUCTION

Chemical Controls

Despite the development of new classes of insecticides and acaricides with novel modes of action which almost certainly will result from extensions of current research, the commerical introduction of such pesticides on a large scale will likely not occur before the year 2000. New pesticide application technology will almost certainly be commercially available before then, however.

Genetic Engineering

The requirement of genetic engineering technology for an indepth biochemical understanding of the traits to be transferred, and the need for biotechnology (tissue and cell culture techniques, plant regeneration techniques etc.) appropriate to the organisms being genetically engineered will delay the commercial introduction of genetic engineering for insect and mite control. The genetic engineering of insect pathogens, such as *Bacillus thuringiensis*, about which a great deal is already known, is likely to result in commercial introduction of new, genetically improved insect pathogens for use in insect management. At least in the case of *B. thuringiensis*, this will likely occur before the year 2000.

Genetic engineering of crop plants for insect and mite resistance is not likely to take place at the commercial level until after the year 2000.

Information Processing

Major advances in information processing hardware and software appropriate for IPM use are continually being made and the necessary biological and ecological monitoring networks are being developed. Major introductions of information processing technology into IPM are underway and will continue at an accelerating pace over the next 20 years. An inadequate biological data base is perhaps the principal limiting factor.

POTENTIAL PRIMARY IMPACTS

Chemical Controls

The primary impact of new classes of insecticides with novel modes of action will be to facilitate insect and mite management by providing additional tools to the pest manager. In addition they will likely have fewer undesirable nontarget effects than currently used insecticides.

New application and formulation technology should reduce nontarget effects and thus contribute to environmental quality. By reducing the quantities of pesticides needed, this technology should reduce the costs of chemical insect control, especially when used in broadly based IPM programs.

Genetic Engineering

Ultimately, genetic engineering should result in a major increase in the use of insect pathogens in IPM. It should also lead to a major increase in use of insect resistant crop varieties in IPM programs.

Information Processing

Information processing technology will have a major impact on insect and mite management, as it will on all of agriculture. It will make large amounts of relevant information and more up-to-date information available to farmers on an as needed basis to facilitate planning and implementation of pest management programs. Its major impact will be to facilitate pest management decision making and the scheduling of pest management (and other crop production) inputs. The consequences of this will be more profitable crop production.

In each of the areas discussed, emerging technologies will contribute to insect and mite management within the broader context of IPM, and in so doing will make the protection of crops from losses to pests more efficient. Only in the cases of application technology and information processing technology will capital investments be involved. The nature of the emerging technologies is likely to be such that they will have application to IPM on virtually all crops.

REFERENCES CITED

Bowers, W. S. 1983. The search for fourth generation pesticides. pp. 47-58. In: D. L. Whitehead and W. S. Bowers (eds.) Natural Products For Innovative Pest Management. Current Themes in Tropical Science. Vol. 2 Pergamon Press, NY. 586 p.

Bowers, W. S., T. Ohta, J. S. Cleere and P. A. Marsella. 1976. Discovery of insect anti-juvenile hormones in plant. Science, NY. 193:542-547.

Clower, J. P., H. R. Mitchell, D. F. Clower, D. C. Rester, and J. B. Graves. 1982. Ultra-low volume application of insecticides in vegetable oil. LA Agric. LA Agric. Expt. Stn. 25:22-24.

Croft, B. A. and A. L. Knight. 1983. Evaluation of the PETE phenology monitoring system for integrated pest management of deciduous tree fruit species. Bull. Entomol. Soc. Amer. 29:37-42.

Davidson, R. H. and W. F. Lyon. 1979. Insect pests of farm, garden, and orchard. 7th Edition. J. Wiley & Sons, NY. 596 p.

Falcon, L. A. 1976. Problems associated with the use of arthropod viruses in pest control. Ann. Rev. Entomol. 21:305-324.

Gage, S. H., M. E. Whalon, and D. J. Miller. 1982. Pest event scheduling system for biological monitoring and pest management. Environ. Entomol. 11:1127-1133.

Glass, E. H. (ed.) 1975. Integrated pest management: rationale, potential, needs and implementation. Entomological Society of America, Special Publication 75-2. 141 p.

Harris, M. K. (ed.) 1980. Biology and breeding for resistance to arthropods and pathogens in agricultural plants. Texas Agric. Exp. Stn. Pub. MP-1451. 605 p.

Hedin, P. A. (ed.) 1983. Plant resistance to insects. ACS Symposium Series 208. American Chemical Soc. Wash., D.C. 375 p.

Huffaker, C. B. (ed.) 1980. New technology of pest control. J. Wiley & Sons. NY. 500 p.

Lane, M. D. and S. E. Law. 1982. Transient charge transfer in living plants undergoing electrostatic spraying. Trans. ASAE. 25:1148-1159.

Ladd, T. L., Jr. and D. L. Reichard. 1980. Photoelectrically operated intermittent sprayer for the insecticidal control of horticultural insect pests. J. Econ. Entomol. 73:525-528.

Ladd, T. L., Jr., D. L. Reichard and D. E. Simonet. 1981. Integration of a photoelectrically operated intermittent sprayer with action thresholds for control of lepidopterous pests of cabbage. J. Econ. Entomol. 74: 698-700.

Ladd, T. L., Jr., D. L. Reichard, D. L. Collins, and C. R. Buriff. 1978. An automatic intermittent sprayer: a new approach to the insecticidal control of horticultural insect pests. J. Econ. Entomol. 71:789-792.

Law, S. E. 1978. Embedded electrode electrostatic induction spray-charging nozzle: theoretical and engineering design. Trans. ASAE. 21:1096-1104.

Law, S. E. 1981. Electrostatic deposition of pesticide spray onto foliar targets of varying morphology. Trans. ASAE. 24:1441-1448.

Law, S. E. and M. D. Lane. 1980. Electrostatic deposition of pesticide sprays onto ionizing targets: charge- and mass-transfer analysis. IEEE (IAS) Record. 2:977-983.

Levin, B. R. 1979. Problems and promise in genetic engineering in its potential applications to insect management. p. 170-175. In: M. A. Hoy and J. J. McKelvey, Jr. (eds.) Genetics In Relation To Insect Management. The Rockfeller Foundation, NY. 179 p.

Manley, D. G. 1982. Use of an electrostatic sprayer for cotton insect control. J. Econ. Entomol. 75:655-656.

Marchant, J. A. and R. Green. 1982. An electrostatic charging system for hydralic spray nozzles. J. Agric. Engng. Res. 27:309-319.

Maxwell, F. G. and P. R. Jennings. (eds.) 1980. Breeding plants resistant to insects. John Wiley & Sons. NY. 683 p.

Menn, J. J. 1983. Present insecticides and approaches to discovery of environmentally acceptable chemicals for pest management. pp. 5-31. In: D. L. Whitehead and W. S. Bowers (eds.) Natural Products For Innovative Pest Management. Pergamon Press, NY. 586 p.

Metcalf, R. L. 1975. Insecticides in pest management. pp. 235-273. In: R. L. Metcalf and W. H. Luckman (eds.) Introduction To Insect Pest Management. John Wiley & Sons. NY. 587 p.

Metcalf, R. L. 1980. Changing role of insecticides in crop protection. Ann. Rev. Entomol. 25:219-256.

Metcalf, R. L. and W. H. Luckman (eds.) 1975. Introduction to insect pest management. John Wiley & Sons. NY. 587 p.

Nordlund, D. A., R. L. Jones and W. J. Lewis (eds.) 1981. Semiochemicals: their role in pest control. John Wiley & Sons. 306 p.

Painter, R. H. 1951. Insect resistance in crop plants. Univ. Press of Kansas. Lawrence. 520 p.

Pimentel, D., J. Krummel, D. Gallahan et al. 1978. Benefits and costs of pesticide use in U.S. food production. BioScience. 28:772-784.

Pryce, R. J. 1983. The search for new agrochemical leads from natural sources. pp. 73-90. In: D. L. Whitehead and W. S. Bowers (eds.) Natural Products For Innovative Pest Management. Current Themes in Tropical Science. Pergamon Press, NY. 586 p.

Rabb, R. L. and F. E. Guthrie (eds.) 1970. Concepts of Pest Management. North Carolina State University Graphics, Raleigh. 242 p.

Reichard, D. L. and T. L. Ladd, Jr. 1983. Pesticide injection and transfer systems for field sprayers. Trans. ASAE. 26.

Reichenberger, L. 1980. Chemical application: the billion-dollar blunder. Successful Farming. 78:23-27.

Roberts, D. W., R. A. LeBrun and M. Semel. 1981. Control of the Colorado potato beetle with fungi. pp. 119-137. In: J. H. Lashomb and R. Casagrande (eds.) Advances in Potato Pest Management. Hutchinson Ross Publishing Co., Stroudsburg, PA. 288 p.

Rosenthal, G. A. and D. H. Janzen (eds.) 1979. Herbivores: Their Interaction With Secondary Plant Metabolities. Academic Press, NY. 718 p.

Sill, W. H., Jr. 1982. Plant protection: an integrated interdisciplinary approach. Iowa State Univ. Press, Ames. 297 p.

Smith, R. F. 1970. Pesticides: their use and limitations in pest management. p. 103-113. In: R. L. Rabb and F. E. Guthrie (eds.) Concepts of Pest Management. North Carolina State University Graphics, Raleigh. 242 p.

Tinsley, T. W. 1979. The potential of insect pathogenic viruses as pesticidal agents. Ann. Rev. Entomol. 24:63-87.

Verloop, A. and C. D. Ferrell. 1977. Benzolphenylureas - a new group of larvicides interfering with chitin deposition. pp. 237-270. In: J. R. Plimmer, P. C. Kearny, G. Kohn, J. J. Menn and S. K. Ries (eds.) Pesticide Chemistry in the 20th Century. A.C.S. Symposium Ser. No. 37. Amer. Chem. Soc., Wash., D.C.

Welch, S. M. 1984. Developments in computer-based IPM extension delivery systems. Ann. Rev. Ent. 29:359-381.

Welch, S. M., B. A. Croft, J. F. Brummer, and M. F. Michels. 1978. PETE: An extension phenology modeling system for management of multi-species pest complex. Environ. Entomol. 7:487-494.

Whitehead, D. L. and W. S. Bowers (eds.) 1983. Natural products for innovative pest management. Current Themes in Tropical Science. Pergamon Press, NY. 586 p.

Background Paper No. 14

Biological Nitrogen Fixation

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BIOLOGICAL NITROGEN FIXATION
Summary

Nitrogen is a critical nutrient for crop production. Although abundantly available--either as atmospheric nitrogen (N_2) or in organic complexes in the soil--nitrogen in these forms cannot be used directly by plants. It must first be changed to ammonia (NH_3) or nitrate (NO_3^-). Thus the large supply of nitrogen needed to grow crops is most commonly provided by nitrogen fertilizers. However, such fertilizers are expensive, and their production consumes a nonrenewable resource, hydrocarbons.

Nitrogen can also be provided through biological nitrogen fixation, a process by which certain bacteria and blue-green algae use an enzyme, nitrogenase, to convert N_2 to NH_3. The most important of these bacteria agriculturally belong to the geneus Rhizobium. These bacteria enter the roots of legumes and form nodules in which they "fix," or convert, nitrogen in the air to forms used by plants. A legume may receive all of its nitrogen needs this way, given the right Rhizobium. In turn, the rhizobia are somewhat protected from microbial competition and predation and from other detrimental effects in the soil environment.

Other kinds of nitrogen-fixing bacteria live near cereal crops and grasses, possibly providing small, beneficial amounts of nitrogen to the plants and receiving needed organic compounds but no protection from detrimental effects in return. This relationship is known as associate fixation.

If its magnitude can be increased, the process of biological nitrogen fixation offers an attractive way to supply the large nitrogen demand of crops without the extensive use of nitrogen fertilizers. To this end, considerable research has been done in the last decade on the biochemistry and genetics associated with the process, and much useful information has been gleaned from this basic research. Research is also under way to determine the possibility of developing cereal crops that fix their own nitrogen, and recent studies have provided needed approximations of the amount of nitrogen provided by associative fixation.

To provide enough nitrogen biologically to sustain high crop yields, however, the stresses affecting legumes and Rhizobium must be better understood, and improved bacterial strains and other ways to overcome these constraints must be found. These developments will come from a combination of well-established techniques and agronomic practices as well as new technologies. For example, conventional strain selection and genetic manipulation may be used to produce strains of rhizobia that can compete with soil microorganisms or that can resist abiotic stresses such as pesticides, drought, and high temperatures. Plant breeding will be used to develop legumes that are better acclimated to soil conditions, have greater photosynthetic activity and less photorespiration, can resist nodulation by less effective soil rhizobia in favor of inoculated rhizobia, and can prolong the duration of fixation. Less likely to come to fruition in this century, but of great importance, will be the development of cereals that can fix their own nitrogen in their tissues or root zones.

If funding is adequate, greater nitrogen fixation from legume-bacterial symbiosis will be realized in the next 10 years, and that from the associative fixation of cereal roots will be realized in 15 years. The benefits of these and future improvements will be the reduced use of hydrocarbons for fertilizer production, an increase in the availability of fertilizer worldwide, and less contamination of ground water.

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BIOLOGICAL NITROGEN FIXATION

Introduction

Of the elements that limit crop production in agricultural land and often plant growth in natural ecosystems, nitrogen stands out because of the large plant demand for it and because its insufficient supply is often the dominant factor preventing the attainment of high yields. However, nitrogen is present in abundant supply, either as N_2 in the atmosphere or as organic complexes in soil. Because these vast supplies cannot be used directly by plants, the need for this critical nutrient for crop production is answered by the use of fertilizers, but nitrogen fertilizers are expensive and their production consumes a nonrenewable resource, hydrocarbons.

Biological nitrogen fixation can provide plants with nitrogen in a form that they can use. The quantity of nitrogen presently added to soil by this process is generally insufficient to sustain high plant productivity and large crop yields. Because of the enormous need for nitrogen for crop production, considerable research has been done in the last decade to understand the biochemistry and genetics of the process and to devise means to increase biological nitrogen inputs to agricultural systems.

In biological nitrogen fixation, atmospheric nitrogen (N_2) is converted to forms used by living organisms. The enzyme bringing about this reaction is known as nitrogenase. Nitrogenase is present only in certain microorganisms, specifically certain baceria and blue-green algae. These microorganisms may live in symbiosis with higher plants or they may live apart, to a lesser or greater extent, from plants. Of the bacteria that are significant in agriculture, the most important are members of the genus Rhizobium. These soil bacteria enter directly into roots of legumes, where they form swellings known as nodules. In the nodule, the bacteria are often, but not always, able to convert atmospheric nitrogen into forms used by legumes. A legume may be able to get all of the nitrogen it needs from the actions of the right Rhizobium in its root nodules. The rhizobia present in this structure benefit also because they are protected from competition, predation and other detrimental effects arising from activities of microorganisms in the surrounding soil.

Microorganisms of the genus Frankia invade the roots of certain woody plants and induce the formation of nodules that also contain nitrogenase. This symbiosis is not significant for the production of food for humans or feed for animals in the United States.

Blue-green algae grow readily in many flooded soils used for growing rice. The nitrogen fixed by these free-living algae is sufficient for low yields of rice. Also prevalent in rice fields of southeast Asia is a fern, Azolla, which contains a blue-green alga. The fern-alga symbiosis may grow rapidly under conditions prevailing in rice fields in Asia and cause large increases in the nitrogen content of the flood water. This two-membered association is exploited to provide nitrogen to paddy rice in areas of China, Viet Nam and other countries.

Associated with the roots of certain cereal crops and other grasses are bacteria that fix nitrogen. The plant excretes organic compounds that the bacteria use, and the bacteria provide nitrogen to the plant, usually in quite small amounts. These bacteria are not in nodules, and many are thus not protected from harmful interactions with microorganisms in the soil. This plant-bacterium relationship has been designated "associative fixation."

The legume-Rhizobium symbiosis is the major contributor to nitrogen fixation in current agricultural systems. In fields planted to soybeans that bear nodules induced by the appropriate bacteria, much of the nitrogen used by the crop comes from the microbial nitrogenase. There is also considerable nitrogen fixation during the growth of legumes used for forage or green manure. In Asian agriculture, blue-green algae and Azolla are significant contributors to the nitrogen economy of the rice field. The large demand of cereal crops for fertilizer nitrogen and direct analytical measurements of the reaction, however, show that there is not appreciable fixation by the bacteria present in the root zone of these crops.

Research is currently directed to improving nitrogen fixation by the legume-Rhizobium symbiosis by developing more active strains of Rhizobium and varieties of legumes. Less attention is being given to developing Rhizobium strains more able to cope with stresses that deleteriously affect the bacteria. Because of the critical role of cereals in food and feed production and the enormous amount of fertilizer nitrogen applied to cereal crops, considerable research is being performed on the biochemistry and genetics of the organisms that may be exploited, directly or indirectly, to increase the amount of nitrogen provided biologically to cereals. Some of this research has the ultimate goal of developing cereals that have the capacity to fix their own nitrogen. Far less attention in the United States is given to the nitrogen fixers that are, or may be, important in rice cultivation.

Current State of the Art

Improved Strains of Rhizobium

Farmers regularly report benefits when they inoculate seeds of legumes with Rhizobium if such bacteria are absent from soil or when indigenous soil rhizobia fix little nitrogen in symbiosis with the plants. To provide the farmer with the correct inocula, considerable research and development has been done, initially in agricultural experiment stations and, in recent years, in private industry. As a result, the technology of producing inoculants has greatly advanced. However, with a few exceptions, the inoculum strains have been selected only on the basis of their ability to fix nitrogen and their capacity to compete with other rhizobia under controlled conditions. Although it is known that a strain highly active under controlled conditions often does not fix much nitrogen in the field and that competition among rhizobia is not the only stress on these bacteria prior to their invasion of the root, little progress has been made in obtaining improved strains based on other selective criteria.

Genetic techniques have been applied in a few instances to obtain improved strains. Thus, Maier and Brill (1978) used mutants of Rhizobium japonicum that nodulated soybean roots more quickly than the original strain and were better nitrogen fixers under greenhouse conditions. Because energy is a major limitation, improved strains may be developed by increasing the amount of available energy. One means of doing so is to use rhizobia that evolve less H_2, the evolution of this gas representing a loss of energy to the symbiosis. Mutants with an altered H_2 metabolism may represent a promising way to increase the yield and nitrogen content of legumes (Hanus et al., 1981).

Much research is currently being conducted on the genetics and biochemistry of this bacterium. Most of these studies are concerned with the nitrogen-fixing process or energy metabolism because it is believed that improvement of these activities has the greatest likelihood of yielding improved strains of *Rhizobium* for use in the field.

Stress-Tolerant Rhizobium

Inoculation with *Rhizobium* strains that actively fix nitrogen in greenhouse-grown legumes and with strains that compete well with other strains of *Rhizobium* should give good results in the field, but frequently the responses are disappointing. These poor responses in field tests probably result from stresses in nature that restrict the growth or survival of the currently available, active nitrogen-fixing bacteria. *Rhizobium* may not endure after its application to seeds, it may not persist in soil, it may not compete with the large numbers of bacteria (other than *Rhizobium*) also growing on the root, it may be consumed by protozoan predators, it may not grow or survive in acid soils, it may be reduced in number as soils dry, or it may be inhibited by fungicides used for pest control (Alexander, 1982).

Several approaches have been devised to counter these stresses. For example, poor survival on seeds may be minimized by entrapment of the bacteria in a polyacrylamide gel (Dommergues et al., 1979). Because strains differ markedly in the rates of their decline on inoculated seed (Philpotts, 1977; Osa-Afiana and Alexander, 1982), their capacity to form nodules or to survive in acid environments (Munns et al., 1979; Lowendorf et al., 1981), and their tolerance to desiccation in soil (Osa-Afiana and Alexander, 1982), the better strains could be used to make inocula. To minimize or overcome the detrimental effect of fungicides applied to seeds, chemicals with a minimum of effect on the nodulating organism could be used (Curley and Burton, 1975), the treated seeds could be coated with a protective material (Diatloff, 1970) or fungicide-resistant mutants could be added as inocula (Odeyemi and Alexander, 1977).

Legumes More Active in Nitrogen Fixation

Little attention has been given to selection of host cultivars to increase nitrogen fixation. However, varieties of the same legume species vary enormously in their capacity to support nitrogen fixation in connection with the bacterial symbionts, so that selection and subsequent breeding will yield improved varieties. Although major breakthroughs can be achieved more readily in bacterial genetics than in plant genetics, the significance of the plant genome in regulating the rate and extent of nitrogen fixation makes more extensive work in this area imperative.

A number of factors in the legume symbiosis are under genetic control by the plant. These factors include nodule formation and the rate and extent of nitrogen fixation. Recent investigations have yielded lines of plants that are nonnodulating or that form ineffective nodules, established how these characteristics are inherited, and categorized the structural and physiological expression of these traits (Vance et al., 1984). Early studies were done with clovers. More recent research has focused on soybeans, alfalfa and peas. In soybean, for example, genes associated with nonnodulating plants have been characterized. These traits are usually dominant, but recessive genes occasionally are involved (Devine, 1984). Five genes in alfalfa determine the formation of ineffective nodules (Vance et al., 1984). The genetic characteristics of mutant lines of peas also have been defined (Holl, 1975). These preliminary studies suggest that genetic manipulation offers considerable promise for increasing nitrogen fixation.

Root Zone of Cereals

Because cereals represent the cornerstone of American agriculture, much effort has been devoted to seeking means to create nitrogen-fixing cereals or to enhance the activity in the rhizosphere of grasses. The rhizosphere contains an enormous number of bacteria that live by using the organic compounds excreted through the root system. Studies in the early 1960's of tropical grasses indicated that the root zones of some contain large numbers of bacteria which, when tested in the laboratory in bacteriological media, brought about appreciable nitrogen fixation. These findings prompted further studies with cereals used for food and feed. The initial observations suggested considerable nitrogen-fixing activity associated with roots. However, much of that initial information was derived from studies of roots removed from soil and then incubated prior to assays of activity, and subsequent inquiry revealed that the bacteria multiplied during this pre-incubation period, thereby giving incorrect values for extrapolation to natural conditions. The current consensus is that the amount of nitrogen fixed and provided to the cereal or other grass is usually small. Nevertheless, the extent of fixation may be sufficiently important to be of economic value, at least in the tropics (Dobereiner, 1982). Apart from flooded soils planted to rice, this associative fixation appears to occur sporadically, the variation among experiments is high and the rates may be inadequate to meet the needs for intensive cropping (Evans and Barber, 1977). Nevertheless, inoculation with active bacteria or the use of appropriate plant varieties may allow for appreciable nitrogen gains. Recent information, for example, indicates that Azospirillum inoculants may increase the yield of corn, wheat, sorghum and forage grasses, although the beneficial effects may result not from nitrogen fixation but rather from a larger or more efficient root system or greater nutrient uptake (Dart, 1984; Okon, 1984). Developing new plant varieties may be a fruitful direction for practical exploitation inasmuch as genetic lines of some cereals and forage grasses differ in the extent of nitrogen fixation supported around their roots (Silver and Hardy, 1976).

It is possible that plants can be modified genetically so that they themselves bring about nitrogen fixation. This approach relies on the introduction into plants of DNA bearing the genetic information to bring out nitrogen fixation, the integration of that genetic information into the plant genome and the phenotypic expression of these genetic characteristics. Accomplishing this goal requires considerable exploratory research, but the significant progress made to date suggests that the approach may be feasible. Much has been learned about the genetics of free-living nitrogen fixers, information that is needed if means ultimately are to be found to transfer the genes for nitrogen fixation into plants. Nitrogen-fixing genes have been transferred into bacteria that themselves have no such capacity. The genetic information that was transferred by plasmids (nonchromosomal cell constitutents containing DNA) was then expressed so that the recipient cell could use N_2 (Brill, 1978). Transfer of the nitrogen-fixing genes from bacteria to higher plants requires a vector of the bacterial DNA. This vector could be plasmids of the bacterium Agrobacterium because its plasmids are inserted into the genome of higher plants in nature. Other means that are being considered for inserting bacterial DNA into plants include the use of cauliflower mosaic virus and chloroplasts. It has also been reported that genes of a bacterium can be transferred to baker's yeast, but the nitrogen-fixing characteristic coded by these genes is not expressed.

The fusion of protoplasts (cells from which the walls have been removed) is another way of introducing nitrogen-fixing genes into higher plants. Protoplasts can be prepared by exposing plant cells or tissues to enzymes that degrade components of the cell walls, and these protoplasts incorporate nucleic acids and bacteria. Initial studies suggest that plant protoplasts can take up nitrogen-fixing organisms (Burgoon and Bottino, 1977).

Flooded Rice

Blue-green algae often become abundant in flooded fields planted to rice, and these microorganisms may fix considerable nitrogen (App et al., 1980; Roger and Kulasooriya, 1980). Indeed, in subsistence agriculture in Southeast Asia, much or essentially all of the nitrogen that is removed from the field as harvested grain may be returned by the activities of blue-green algae. The rate and extent of fixation depend upon the alga that spontaneously appears in the flooded field, and about 30 pounds of nitrogen may be added per acre per year in Asian conditions (Watanabe et al., 1977). However, the algae that flourish spontaneously in the field may not be able to fix nitrogen or may have low activity, and hence inocula are sometimes added to the water. These inocula may promote an increase in rice yield (Roger and Kulasooriya, 1980). Fixation by algae in flooded rice fields has been the subject of considerable study in Asia, but little attention has been devoted to this process for U. S. rice production.

The fern Azolla bearing its blue-green algal symbiont is used to enrich paddy fields in China and Viet Nam with nitrogen, and research on this subject is underway in the Philippines, Sri Lanka and the United States. Because the fern grows in the water, it can be cultivated at the same time as rice, thereby adding nitrogen during the flooded period. Because of the considerable nitrogen that may be added to the field and the compatibility of fern growth with rice production, the area in Southeast Asia in which Azolla is being cultivated has increased markedly in recent years (Lumpkin and Plucknett, 1982). Studies in Indonesia also indicate that rice yield is increased when this fern association is introduced into the cropping system, and such additions supplement or substitute for nitrogen fertilizer added for the growth of rice (Brotonegoro et al., 1982). Studies in the United States also show that incorporating the fern into soil of fallow fields increases the yield of paddy rice. However, existing methods of culturing the fern require considerable labor, and the cost of labor makes it unlikely that present means of handling Azolla are attractive for the highly mechanized means of growing rice in this country (Talley et al., 1981).

Major Breakthroughs Needed

Rhizobium

For the immediate future, the chief source of biologically fixed nitrogen in agriculture will be the legume-Rhizobium symbiosis. Because of the major breakthroughs in bacterial genetics, it is likely that significant strides can be made by Rhizobium strain development as well as by conventional strain selection. However, modern genetics has only occasionally been applied to developing improved rhizobia. The improvements that have, in fact, been made to date have come from conventional strain selection. Further information is needed to define the factors that affect Rhizobium in soil, during its colonization of the rhizosphere and when functioning in the nodule. Nitrogen-fixing activity is an essential trait but is not the only characteristic needed for improved rhizobia. This is evident from the frequent observation that this bacterium, although fixing at high rates when tested on legumes grown in greenhouses, fails to fix much nitrogen in the field. With information on the significant ecological and physiological stresses that, in nature, affect the survival, growth and activity of these organisms, improved bacterial strains can be obtained, either by conventional strain selection or by genetic manipulation; such useful traits may be introduced into the bacteria by means of plasmids or techniques involving recombinant DNA.

A major constraint affecting rhizobia is the community of microorganisms in soil. These organisms may prevent Rhizobium from becoming established in large numbers because of competition, or they may prey on the root-nodule bacteria and markedly reduce their numbers. An introduced Rhizobium may also encounter competition with soil rhizobia that have poor or no nitrogen-fixing activity. Were these stresses to be overcome, then the improved strains of Rhizobium would form a higher percentage of nodules on legumes in the field and thus bring about greater nitrogen gains. The extent of the suppression by other organisms is evident from reports that only 5 to 10% of the nodules on soybeans may originate from the added inoculum strain (Devine, 1984). The poor ability of Rhizobium to colonize roots even of their host legumes is also evident in the benefit arising from large as compared to small inocula (Holland, 1970).

Rhizobia grow slowly, and they also use little of the organic matter in soil. In the rhizosphere, the readily available nutrients are used more rapidly by other bacteria so that rhizobia are not dominant in the microbial community of soil or the rhizosphere. Although strain selection has been used to obtain rhizobia that are more competitive with members of the same genus, even these strains often fail to occupy most of the nodules on inoculated legumes in the field. Means must thus be found to overcome the competition that restricts the proliferation of these organisms in soil and around growing roots.

Rhizobium appears to be quite susceptible to predation by the protozoa that proliferate in the rhizosphere. The protozoa feed simultaneously on the dominant bacteria and also on the smaller numbers of root-nodule bacteria. Means to minimize such attack by protozoa need to be devised (Alexander, 1982). Predation by protozoa and competition with other soil microorganisms and with indigenous rhizobia may be overcome by introducing into rhizobia, possibly by plasmid technology, the capacity to produce antibiotics that inhibit the organisms detrimental to the inoculum strain (National Academy of Sciences, 1982). Another approach is to apply, either to seeds or to foliage, chemicals that inhibit indigenous rhizobia, other competitors or predatory protozoa; if the inoculum strain is made resistant to the introduced chemicals, its growth in the rhizosphere will be enhanced by the presence of the chemicals.

Rhizobium is sensitive to various abiotic stresses. These stresses include drying of the seeds on which the inoculum is placed, the acidity common in many soils in which legumes are grown, the drying of soils, the high temperatures that are occasionally encountered in nature and pesticides applied to seeds to control pests (Munns et al., 1981; Alexander, 1982). Means need to be found to overcome those abiotic stresses which are important in particular legume-growing areas.

It is often desirable to have rhizobia that persist and maintain high numbers in soil so that the roots of subsequent crops of legumes will encounter large populations of effective bacteria. However, many of the introduced strains fail to persist in appreciable numbers. Rhizobia thus need to be obtained that survive well in nature. For this purpose, either the natural variability in persistence among the rhizobia or laboratory-induced modifications can be the basis for getting improved inocula.

Rhizobia infect, nodulate and fix nitrogen with only a limited number of legume hosts. If it is deemed desirable to have strains that can infect and fix nitrogen in association with many legume hosts, such promiscuous bacteria need to be devised using modern genetic techniques.

As strains are obtained or developed that can overcome these biotic and abiotic stresses, genetic techniques should be used to obtain rhizobia that fix even more nitrogen and do so at lower energy costs. Progress has been made in this direction in the finding of bacteria that have an altered metabolism of H_2 so that energy is not wasted by H_2 evolution. Bacteria should be developed that nodulate and continue to fix nitrogen actively in the presence of fertilizer nitrogen or of ammonium or nitrate generated microbiologically in the soil, inasmuch as a plant that nodulates and nodules that continue to fix nitrogen in soils containing much inorganic, fixed nitrogen would be of great benefit.

Legumes

It is often the plant and not the rhizobium that determines the rate and extent of fixation, and hence additional agronomic and genetic research is needed on the plant component of the two-membered association. Major breakthroughs will come from plant-breeding, sophisticated genetic techniques and straightforward agronomic approaches.

Both soil and plant constraints affect legume growth. The constraints include dryness, acidity, nutrient deficiencies, poor soil structure and salinity (Munns and Franco, 1982). Means need to be devised to overcome these frequently major obstacles to high yield. Plant breeding may provide legumes that are able to overcome some of the limitations on nitrogen fixation expressed through the plant. Aluminum toxicity associated with acid soils may also be overcome by developing tolerant varieties; some work in this direction is in progress. In semi-arid rangelands, greater nitrogen fixation may be achieved by introducing legumes that are adapted to these circumstances (Johnson et al., 1981). The existence of genetic variability among plants in tolerance to salinity may be used to obtain varieties that are able to grow in saline soils (Marcarian, 1982). Often breakthroughs will be achieved by conventional agronomic approaches to intercropping and crop rotation; hence, additional information is needed on intercropping and the best rotations for modern crop production.

The high yields and improved quality of legumes, to a large degree, are the result of plant breeding. Research is needed, however, on developing cultivars that are more active in nitrogen fixation. One way is to prolong the period that legumes continue to support nitrogen fixation. Because the rate of fixation in the nodules of many legumes declines markedly after flowers appear, considerable benefit would be gained if the symbiosis would fix nitrogen for more of the growing season; breeding is an ideal way to provide such plants to the farmer.

The supply of energy from photosynthesis is a major limitation to nitrogen fixation. Plant breeding should be successful in developing legumes with greater photosynthetic activity, and the additional carbon translocated to the root nodules of such varieties should sustain greater nitrogen gains by the symbiosis (Evans and Barber, 1977). Plants with a reduced rate of photorespiration and with lower levels of root respiration may also have more energy from photosynthesis available for nitrogen fixation (Gibson, 1976). The inhibition of nodulation and nitrogenase activity by fixed nitrogen reduces the extent of fixation, and hence the selection or development of plants with a diminished capacity for assimilation of combined nitrogen from the soil should be beneficial. Still another useful approach is to breed for legumes that exclude nodulation by indigenous soil rhizobia so that the roots will be invaded only by the introduced, highly active strains derived from inoculants (Devine, 1984).

Cereals

Major breakthroughs in developing cereals that have nitrogen-fixing activity in the root zone or in their tissues will take time. Considerable research is necessary to determine the feasibility of these approaches and, if feasible, to develop the desired plants and cropping systems. Nevertheless, the importance of cereal crops to agriculture dictates that a commitment be made to maintaining this long-term research. Considerable information in basic science needs to be obtained before there will be significant economic return from nitrogen-fixing cereals. Because plants do not fix nitrogen and few support much nitrogen fixation in their rhizospheres, the evolutionary pathway leading to the emergence of such plants is difficult, even though they would have an enormous selective advantage in nature.

The major steps needed to develop a plant that fixes nitrogen are (a) the transfer of genes for nitrogen fixation from an organism possessing those genes into the cells of the plant, (b) the replication of that foreign DNA, (c) the physiological expression of the genetic capability for nitrogen fixation and (d) the regeneration of intact plants from the cells or tissues which have the nitrogenase activity. DNA can be transferred from bacteria into plant cells by means of chloroplasts, a bacterial (Agrobacterium tumefaciens) plasmid that enters plant cells or the cauliflower mosaic virus (Brill and Ela, 1981). If the enzyme system is successfully transferred and is expressed, it must be supplied with considerable energy in the form of products from photosynthesis. Because photosynthesis often limits growth, the yield of the newly created nitrogen-fixing cereal might be reduced owing to the energy demand of the enzymes metabolizing N_2. Hence, it will be necessary to increase the efficiency of photosynthesis in order to satisfy the new demands created by genetic engineering. Success in this endeavor also requires that the plant cells possess a means for protecting the nitrogenase enzyme system from inactivation by O_2. The sensitivity of this enzyme system to O_2 is overcome in legumes and free-living bacteria by a variety of mechanisms, each of which is essential in order to have a functional reaction. Means have been proposed to overcome the O_2 inhibition in genetically engineered organisms, but none has been tried (Dixon, 1978). Means will also have to be devised to regulate the metabolism of the new organism in order that adequate nitrogen is acquired to satisfy the new plant but that not too much N_2 is converted to ammonia, the end product of the initial fixation reaction. Ammonia could be toxic, and its formation would also consume considerable energy, neither of which is desirable.

Major breakthroughs are needed if associative fixation is to be of practical significance. One approach involves the breeding of plants that support greater activity in the rhizosphere. This enhanced activity will probably require a greater quantity of carbon to be excreted through the roots. Plant breeding to achieve these ends is likely to be successful because there are appreciable differences in varieties of a number of cereal crops and forage grasses in the activity of the nitrogen fixers developing around or associated with the roots (Diem and Dommergues, 1980; Brill and Ela, 1981). Another approach is to obtain, either by conventional selection methods or by genetic engineering, bacteria that are more active in nitrogen fixation (Okon, 1984). This approach is not as simple as it might seem because significant nitrogen accretion requires extensive growth of the microorganism, but such proliferation of the initially small population added as an inoculum necessitates having bacteria able to cope with numerous competitors, many of which grow quickly, as well as protozoa that prey on bacteria. Means to overcome competition and predation in the rhizosphere do not presently exist, but it should be possible to favor the inoculum organism in some selective way; for example, by giving it the capacity to grow quickly or to excrete compounds inhibitory to competing bacteria or grazing protozoa.

The final phase will be the development of an appropriate crop management system. The environmental constraints, the nutrient needs and the pests affecting the new cereal varieties will have to be defined, and a crop management system will need to be devised for high yields. Because methods now exist for developing management systems for new varieties, no major breakthroughs in the area are required.

Flooded Rice

The chief breakthroughs needed to exploit the *Azolla* symbiosis include selecting species or strains that are efficient in using low levels of phosphorus, that tolerate temperature extremes, and that are resistant to pests that appear in the paddy field

(National Academy of Sciences, 1982). Iron-scavenging species may be needed because iron deficiency may restrict development of the fern at high pH values. Control of spore formation by the ferns would be useful for breeding new varieties and to serve as a source of inoculum, particularly in view of the difficulties in working with vegetative ferns. However, the chief limitation is the high labor costs and the absence of equipment for mechanically handling the ferns, and hence their use for rice production in the United States is presently unattractive.

Although nitrogen-fixing blue-green algae can fix moderate amounts of nitrogen in paddy fields, the selection of species that develop well at low phosphorus levels and, in waters of high pH, at low iron levels should permit greater nitrogen gains when these nutrient elements are limiting. These microorganisms often suffer from competition by non-fixing species, both blue-greens and other algae, and means need to be found to minimize this competition. Furthermore, blue-green algae are often voraciously consumed by the aquatic invertebrates that occasionally proliferate extensively in the water, and means will need to be found to overcome such grazing (Alexander, 1982). Nevertheless, blue-green algae are not presently considered to be important as nitrogen sources for U.S. agriculture, although they are significant in tropical and subtropical regions of Asia and Africa.

Institutional Arrangements

Agricultural Experiment Stations

Much of the available information on the utility of the legume-Rhizobium symbiosis in U. S. agriculture comes from research done during the past 80 years at agricultural experiment stations. These studies have involved crop and soil scientists and microbiologists. These experiment stations have personnel skilled in the laboratory, greenhouse and field disciplines. Such research centers can and should stand at the forefront of future programs in this area. However, as biochemistry, plant physiology and genetics have developed, the earlier multidisciplinary approaches have largely disappeared, and the degree of specialization and extent of knowledge required in individual subjects are such that individual scientists in the experiment stations became largely concerned only with their own disciplines. The chief professional deficiency is thus the paucity or frequent absence of multidisciplinary programs and meaningful interactions among specialists in agronomy, plant physiology, plant breeding, microbiology, genetics and biochemistry. Multidisciplinary approaches are particularly necessary in view of the extent of knowledge required of each scientist in his or her discipline. In this multidisciplinary team, the microbiologists, geneticists, plant physiologists and biochemists should be working together with the more practical and farmer-orientated plant breeders, agronomists and plant pathologists. In this way, the allure of the esoteric areas of research can be combined in a meaningful way with the approaches that will likely provide practical benefit to the agricultural community.

More concern also needs to be given to the agronomic aspects of legume production. Such research has declined with the ever-growing use of fertilizers. Such research should focus on rotations and intercropping involving legumes, agronomic factors limiting their growth and the breeding to obtain better varieties. Additional field testing of legumes and of possible beneficial effects on cereals from associative fixation needs to be performed. Although laboratory and greenhouse studies are critical to establish the relevant aspects of genetics and biochemistry, that information can only be useful when field experiments are carried out to evaluate the actual potential of the crops and cropping systems. The frequency of extensive pursuit of unproductive areas of investigation would be minimized if the laboratory research would soon be followed by greenhouse trials and the promising leads from greenhouse studies would be tested soon after under field conditions.

The increased emphasis on legumes, the greater involvement of crop and soil scientists and the increased concern with field assessments are not to suggest the lack of need for more laboratory studies or for more research on the possibility of developing nitrogen-fixing cereals or associations. This work should continue to be actively pursued. However, the minimum of concern with problems whose resolution will help the farmers in the near term needs to be rectified.

Financial support is limited for research on the agronomic aspects of nitrogen fixation, new approaches using legumes and the development of more active strains of Rhizobium. At the present time, much of the work is supported by small grants from the federal government. However, small grants are not adequate for meaningful, sustained research, and significant breakthroughs will only come when these avenues of investigation receive adequate support for substantive and sustained study.

U. S. Department of Agriculture

The U. S. Department of Agriculture has made a sustained and significant contribution to research on legumes and their nitrogen-fixing activity. This work is continuing at a modest level. However, the program is presently quite small and is limited to several locations in the United States. Moreover, few scientists and technologists are involved. In order to make a more significant contribution and to pioneer in this field, more scientists, additional funding and a broader based program are required. The interactions between federal and state researchers in this area have been good, and it is anticipated that a larger program devoted to both the practical and theoretical aspects of nitrogen fixation will continue to involve both USDA scientists and state experiment station researchers.

Private Universities

Much of the exciting research on the biochemistry and genetics of nitrogen-fixing bacteria has come from private universities. Scientists at these institutions have the interest and competence in biochemistry, genetics, microbiology and plant physiology to continue to provide much useful information. Their continued research is important because they offer a large base of competence in these fields. However, the faculties of private universities generally do not contain the individuals needed for field evaluation and agronomic assessments, and the absence from such institutions of crop and soil scientists, plant pathologists and plant breeders and the difficulties in coordinating research involving private and public universities or USDA dictate that research in these institutions largely focuses on the basic sciences that are critical for major breakthroughs.

Private Industry

Private companies have been the basis for much of the plant breeding in the past, and they will continue to be important in the future. Private industry has also been the source of the Rhizobium inoculants that are presently widely distributed to farmers, and it is likely that the development of inoculant technology and breakthroughs in relevant aspects of plant biotechnology will come from private industry. It is unlikely, however, that there will be the sustained research in industry that will provide the major breakthroughs that are necessary for appreciable progress in this field. Nevertheless, private industry will largely be the source of breeding work, the seeds and microorganisms crucial for future technological developments, and hence their participation in these activities must be encouraged.

Relations Among the Above

Discussions, conferences, cooperative research and other interactions are important among the preceding groups. The basic researchers at universities, experiment stations and USDA need to funnel their findings to the applied researchers at the state and federal experiment stations, and the information that comes from these applied studies needs to be provided immediately to the private companies that will provide seeds and bacteria to farmers. The interchange of information presently occurs, but the exchange of data suffers from the paucity of applied research at state experiment stations and USDA. With the latter need answered, it is expected that the theoretical information will be evaluated promptly under natural conditions, and the important findings from the latter studies will be developed by the private sector to the point that they can be directly useful to American agriculture.

Likely Timing of Commercial Introduction

The timing of introduction of these technologies into agriculture will depend on the extent of financial support. It will also depend on the price of nitrogen fertilizers, and a continuing high cost of fertilizers will stimulate the research, increase the demand for biologically fixed nitrogen and result in an earlier introduction of the relevant technologies. Assuming that adequate financial support is forthcoming for the needed research and development, it is likely that the most significant practical benefits in the short term will come from the legume-Rhizobium symbiosis. If plant breeding programs are extensive, the needed soil and environmental constraints are defined and means to overcome them are developed, greater nitrogen fixation from this symbiosis should be realized in the present decade. This technology requires improved cultivars of plants, information on conditions for maximum fixation, improved strains of Rhizobium and a better inoculant technology. The means to exploit these technologies presently exist, the methods are available and the personnel are well trained.

Characterization of the major constraints affecting Rhizobium, obtaining strains resistant to these stresses or devising technologies to overcome them, and introduction of the strains into commercial inoculant production or the technologies into farm practice can be accomplished within 10 years. Again, the chief question is the extent of funding for this poorly supported activity. The development of strains of Rhizobium better able to cope with natural stresses can often be accomplished by presently available techniques by strain selection or mutation, but genetic engineering may be useful when other techniques are slow or fail.

Much needs to be learned and accomplished before genetic engineering will provide cereals that can fix nitrogen. However, the benefits of successful research in this area are enormous. Specialists in this subject state that the development of nitrogen-fixing cereals "probably will not be achieved in the near future" (Brill and Ela, 1981) or "may not be accomplished during this century" (Dixon, 1978). Even after the genetic engineering is done, moreover, conventional plant breeding must provide the seeds and the needed agronomic evaluations will have to be carried out.

Practical exploitation of associative fixation will also take time. In this instance, however, the process is feasible, but the chief obstacle is the small amount of nitrogen fixed by present root associations. The problems of getting significant fixation include finding plants that excrete carbon without appreciably reducing their own yield, obtaining bacteria that actively fix nitrogen in the rhizosphere and devising means to ensure that these bacteria assume dominance in the face of potent competitors and possible predators. With adequate support for the research, practical benefits may be evident about the year 2000, but the amount of nitrogen gained by associative fixation will still only be a small part of that required by the crop. Nevertheless, even a small increase would be beneficial.

Impacts

The chief impact of greater biological nitrogen fixation will be a reduced demand for fertilizer nitrogen. This, in turn, will diminish the amount of hydrocarbons, a nonrenewable resource, that is consumed for fertilizer production.

Increased crop yields will result from biological nitrogen fixation. However, these yields will be greater only in comparison to unfertilized crops because fertilizers provide the same benefit as the biologically fixed nitrogen.

The reduced demand by U. S. agriculture for fertilizer nitrogen will make fertilizers more available for regions of the world where fertilizers are in short supply. By reducing the demand for fertilizers, the price should also be less.

It is also expected that there will be a reduction in capital needed to produce chemical fertilizers and to make use of fossil fuels. It has been stated that the economic benefits from nitrogen fixation are assumed to be substantial, but these economic benefits have rarely been documented because meaningful economic analyses have rarely been performed (Welsch, 1982). Some consideration has also been given to socio-economic factors, but these factors remain poorly defined (Randolph and Koppel, 1982).

The reduced reliance on fertilizer nitrogen should diminish nitrate pollution of groundwaters and of drinking waters that come from groundwater supplies. A significant part of the fertilizer nitrogen applied in the field is lost to underground waters as nitrate, and the extent of this loss should be diminished with greater reliance on legumes and with the introduction of cereals that have the capacity to make their own nitrogen available.

REFERENCES

Alexander, M., "Research to Enhance Nitrogen Fixation: Misplaced Emphasis?," in Priorities in Biotechnology Research for International Development, pp. 208-229. (Washington: National Academy of Science Press, 1982)

App, A. A., Watanabe, I., Alexander, M. et al. "Nonsymbiotic Nitrogen Fixation Associated with the Rice Plant in Flooded Soils," Soil Sci. 130:293-289, 1980.

Brill, W., "Genetics and Regulation of Nitrogen Fixation," in Limitations and Potentials for Biological Nitrogen Fixation, J. Dobereiner, R. H. Burris, and A. Hollaender, eds., pp. 237-245 (New York: Plenum Press, 1978).

Brill, W. J., and Ela, S. W., "How About a Nitrogen-Fixing Corn Plant?," Microbiology (Washington, D.C.) pp. 396-397, 1981.

Brotonegoro, S., Sudjadi, M., Partohardjono, S. et al., "Some Experiments in the Use of Azolla for Rice Production in Indonesia," in Biological Nitrogen Fixation Technology for Tropical Agriculture, P. H. Graham and S. C. Harris, eds., pp. 567-573 (Cali, Columbia: Centro Internacional de Agricultura Tropical, 1982).

Burgoon, A. C., and Bottino, P. J., "Uptake of the Nitrogen Fixing Blue-Green Alga Gloeocapsa by Plant Protoplasts," in Genetic Engineering for Nitrogen Fixation, A. Hollaender, ed., pp. 213-237 (New York: Plenum Press, 1977).

Curley, R. L., and Burton, J. C., "Compatibility of Rhizobium japonicum with Chemical Seed Protectants," Agron. J. 67:807-808, 1975.

Dart, P. J., "Application of Biological Nitrogen Fixation for Increasing Crop Production," in Advances in Nitrogen Fixation Research, C. Veeger and W. Newton, eds., pp. 45-46 (The Hague: M. Nijhoff, 1984).

Devine, T. E., "Genetics and Breeding of Nitrogen Fixation," in Biological Nitrogen Fixation: Ecology, Technology, and Physiology, M. Alexander, ed. (New York: Plenum Publishing Corp., 1984).

Diatloff, A., "Overcoming Fungicide Toxicity to Rhizobia by Insulating With a Polyvinyl Acetate Layer," J. Aust. Inst. Agric. Sci. 36:293-294, 1970.

Diem, H. G., and Dommergues, Y. R., "Significance and Improvement of Rhizospheric N_2 Fixation," in Recent Advances in Biological Nitrogen Fixation, N. S. Subba-Rao, ed., pp. 191-226 (London: Edward Arnold, 1980).

Dixon, R., "Genetic Engineering in the Fields," New Scientist 78:684-686, 1978.

Dobereiner, J., "Emerging Technology Based on Biological Nitrogen Fixation by Associative N_2-Fixing Organisms," in Biological Nitrogen Fixation Technology for Tropical Agriculture, P. H. Graham and S. C. Harris, eds., pp. 469-483 (Cali, Columbia: Centro Internacional de Agricultura Tropical, 1982).

Dommergues, Y. R., Diem, H. G., and Divies, C., "Polyacrylamide-Entrapped Rhizobium as an Inoculant for Legumes," Appl. Environ. Microbiol. 37:779-781, 1979.

Evans, H. J., and Barber, L. E., "Biological Nitrogen Fixation for Food and Fiber Production," Science 197:332-339, 1977.

Gibson, A. H., "Limitation to Dinitrogen Fixation by Legumes," in Proceedings of the First International Symposium on Nitrogen Fixation, pp. 400-428 (Pullman, Wash.: Wash. State Univ. Press, 1976).

Hanus, F. J., Albrecht, S. L., Zablotowicz, R. M. et al., "Yield and Nitrogen Content of Soybean Seed as Influenced by *Rhizobium japonicum* Inoculants Possessing the Hydrogenase Characteristic," *Agron. J.*, 72:368-372, 1981.

Holl, F. B., "Host Plant Control of the Inheritance of Dinitrogen Fixation in the *Pisum-Rhizobium* Symbiosis," *Euphytica* 24:776-770, 1975.

Holland, A., "Competition Between Soil- and Seed-Borne *Rhizobium trifolii* in Nodulation of Introduced *Trifolium subterraneum*. *Plant Soil* 32:293-302, 1970.

Johnson, D. A., Rumbaugh, M. D., and K. H. Asay, "Plant Improvement for Semi-Arid Rangelands: Possibilities for Drought Resistance and Nitrogen Fixation," in *Soil Water and Nitrogen*, J. Monteith and C. Webb, eds., pp. 279-303 (Hague: M. Nijhoff, 1981).

Lowendorf, H. S., Baya, A. M., and Alexander, M., "Survival of *Rhizobium* in Acid Soils," *Appl. Environ. Microbiol.* 42:951-957, 1981.

Lumpkin, T. A., and Plucknett, D. L. *Azolla as a Green Manure* (Boulder, Col.: Westview Press, 1982).

Maier, R. J., and Brill, W. J., "Mutant Strains of Rhizobium japonicum With Increased Ability to Fix Nitrogen For Soybean," Science 201:448-450, 1978.

Marcarian, V., "Management of the Cowpea/Rhizobium Symbiosis under Stress Conditions, in Biological Nitrogen Fixation Technology for Tropical Agriculture, P. H. Graham and S. C. Harris, eds., pp. 279-286 (Cali, Columbia: Centro Internacional de Agricultura Tropical, 1982).

Munns, D. N., Cassman, K. G., and Keyser, H. H., "Selection of Naturally Occurring Stress Tolerant Rhizobium," in Genetic Engineering of Symbiotic Nitrogen Fixation, J. M. Lyon, R. C. Valentine, D. A. Phillips et al., eds., pp. 181-191 (New York: Plenum Press, 1981).

Munns, D. N., and Franco, A. A., "Some Constraints to Legume Production," in Biological Nitrogen Fixation Technology for Tropical Agriculture, P. H. Graham and S. C. Harris, eds., pp. 133-152 (Cali, Colombia: Centro Internacional de Agricultura Tropical, 1982).

Munns, D. N., Keyser, H. H., Fogle, V. W. et al., "Tolerance of Soil Acidity in Symbiosis of Mung Bean With Rhizobia," Agron. J. 71:256-260, 1979.

National Academy of Science, Priorities in Biotechnology Research for International Development (Washington: National Academy of Science Press, 1982).

Odeyemi, O., and Alexander, M., "Use of Fungicide-Resistant Rhizobia for Legume Inoculation," Soil Biol. Biochem. 9:247-251, 1977.

Okon, Y., "Response of Cereal and Forage Grasses to Inoculation With N2-Fixing Bacteria," in Advances in Nitrogen Fixation Research, C. Veeger and W. Newton, eds., pp. 303-309 (The Hague: M. Nijhoff, 1984).

Osa-Afiana, L. O., and Alexander, M., "Differences Among Cowpea Rhizobia in Tolerance to High Temperature and Desiccation in Soil," Appl. Environ. Microbiol. 43;435-439, 1982.

Philpotts, H., "Survival of Rhizobium trifolii Strains on Inoculated Seed Held at 35°C," Austr. J. Exp. Agric. Anim. Husb. 17:995-997, 1977.

Randolph, R. H., and Koppel, B., "A Technology Assessment of Biological Nitrogen Fixation," in Biological Nitrogen Fixation Technology for Tropical Agriculture, P. H. Graham and S. C. Harris, eds., pp. 665-675 (Cali, Columbia: Centro Internacional de Agricultura Tropical, 1982).

Roger, P. A., and Kulasooriya, S. A., Blue-Green Algae and Rice (Los Banos, Philippines: International Rice Research Institute, 1980).

Silver, W. S., and Hardy, R. W. F., "Newer Developments in Biological Dinitrogen Fixation of Possible Relevance to Forage Production," in Biological N-Fixation in Forage Livestock Systems, C. S. Hoveland, ed., pp. 1-34 (Madison, Wisc.: American Society of Agronomy, 1976).

Talley, S. N., Lim, E., and Rains, D. W., "Application of Azolla in Crop Production," in Genetic Engineering of Symbiotic Nitrogen Fixation, J. M. Lyon, R. C. Valentine, D. A. Phillips et al., eds., pp. 363-384 (New York: Plenum Press, 1981).

Vance, C. P., Heichel, G. H., and Barnes, D. K., "Nitrogen and Carbon Assimilation in *Medicago sativa* Mediated by Host Plant Genes for Nodule Effectiveness," in *Advances in Nitrogen Fixation Research*, C. Veeger and W. Newton, eds., pp. 565-571 (The Hague: M. Nijhoff, 1984).

Watanabe, I., Lee, K. K., Alimagno, B. V. et al., *IRRI Research Paper Series*, 3:1-16, 1977.

Welsch, D. E., "Economic Analysis of Biological Nitrogen Fixation," in *Biological Nitrogen Fixation Technology for Tropical Agriculture*, P. H. Graham and S. C. Harris, eds., pp. 675-681 (Cali, Colombia: Centro Internacional de Agricultura Tropical, 1982).

Background Paper No. 15

Water and Soil-Water-Plant Relations

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WATER AND SOIL-WATER-PLANT RELATIONS

Summary

The distribution of vegetation over the Earth's surface is controlled more by the availability of water than by any other factor. In the United States, agriculture accounts for over 80 percent of the water consumed; about 98 percent of that water is used for irrigation of crops, particularly in the more arid Western States. Several factors complicate the availability of water for irrigation: (1) cities, industry, and farming are in fierce competition for the water available, (2) ground water sources are gradually being depleted, (3) the costs of pumping and distributing surface water are gradually increasing, and (4) many surface and ground waters are being contaminated by a variety of pollutants. Thus techniques to conserve adequate supplies of fresh water have become important.

Many important contributions have been made by studying water requirements of crops. Although this information has helped in planning reservoir and canal sizes, the hope for breeding plants with lower requirements for water has not been realized, and no technologies have been advanced that would help realize this goal in the next 15 years. Nearly all improvements in water use efficiency have come from improved irrigation techniques, especially the timely application of the amount of water needed and application in a manner that minimizes evaporation. (At present, nearly all the water taken up by the plant is immediately passed through and evaporated at the leaf surfaces. Only a very small fraction becomes part of the permanent structure.)

Progress in improving the water use efficiency of crops will hinge on gathering the information needed to develop a theoretical framework of the mechanisms that influence uptake, use, and loss of water--in humid regions as well as arid and semiarid regions. Dramatic progress in the development of instrumentation now permits researchers to measure many plant physiological responses in real time. It also allows the recent measurements of plant hormones and enzymes, which provide additional indications of water stress.

Once the mechanisms of water use efficiency have been identified and a better understanding of the plant as an integrated whole is gained, biotechnology may help in the development of more water-efficient plants. Already, recent experiments suggest that tissue culture may provide material less susceptible to water stress. For example, alfalfa and rice cell lines have been obtained that tolerate 2 percent sodium chloride, a salt concentration lethal to nonselected cells.

For the near term, however, traditional methods of plant breeding must be relied upon, even though there is increasing evidence that for many crops the limits to improvement by this method are being approached. To break through this yield plateau, the breeder must work with the physiologist and biochemist to understand the stress response hierarchy and eventually to control enzymes, membrane characteristics, and mechanisms for communication in the plant.

The technologies available for immediate application are those that prevent losses in transport, particularly those for farm distribution of irrigation water. These include drip irrigation, below-ground distribution of water, deficit irrigation, water harvesting, time and frequency of application, and the forecasting of time and frequency of application.

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TECHNOLOGY, PUBLIC POLICY, AND
THE CHANGING STRUCTURE OF AMERICAN AGRICULTURE

WATER AND SOIL-WATER-PLANT RELATIONS

A. DEFINITIONS AND SCOPE

A.1. Introduction

The availability of fresh water, measured in terms of quantity and place of occurrence, is of utmost importance for a productive agriculture. The availability of water for sustained agriculture has played a decisive role in the development and stability of human societies throughout history. The distribution of vegetation over the surface of the earth is controlled more by the availability of water than by any other single factor.

Regions where crop growth is restricted by water supply have not been able to support the continuous development and growth of complex societies. History records many examples of stagnation and collapse of societies that could not solve their water management problems.

Agriculture accounts for more than 80 percent of the water consumed in the United States, and about 98 percent of that water is used for irrigation of crops. The needs of water for farming, industry, cities, and urban areas are on a collision course. A serious threat exists to the maintenance of adequate supplies of fresh water. Techniques must be developed that conserve water.

An additional serious, but little acknowledged, problem is that of maintaining the quality of water resources. Contamination of surface water and groundwater is of increasing concern in the United States and throughout the world. Remedial actions to clean up bodies of surface water and/or aquifers are difficult, expensive, and mostly not possible.

Water will rapidly become one of the nation's most limiting resources. In several regions the rate of pumping from ground water exceeds the rate of recharge. Some aquifers from which irrigation water or municipal water is drawn are in danger of becoming irreparably damaged by pollution. While the ecological significance of water is becoming recognized, the demand for recreational areas increases, and conflicts between interest groups grow. Water rights and allocation alternatives become important policy issues.

A.2. Subject Matter of Report

The subject matter of this report is water use by plants and the potential for increasing the efficiency of water use by plants, where water use efficiency is defined as mass of carbon dioxide (CO_2) fixed per unit mass of water (H_2O) transpired, e.g. grams of CO_2 fixed per grams of H_2O transpired. The purpose of this report is to identify and define principal lines of research from which major technologies can be developed and made available for commercial introduction by the year 2000.

Water is essential for plant growth. Plant life evolved in the ancient seas during the early geologic history of the Earth. When plants left the aqueous environment, they evolved adaptations to maintain a high water content within their cells, even though the tissues are surrounded by air. Almost every plant process is affected either directly or indirectly by its ability to maintain cell hydration since water serves as a solvent, a reagent, and a transport vehicle.

Terrestrial plants obtain the water they need by absorbing it from the soil through an extensive system of roots. Nearly all of the water that is taken up by the plant is immediately passed through and evaporated at the leaf surfaces. Only a very small fraction of water absorbed becomes part of the permanent structure.

When water supply is not adequate, many plant processes are affected adversely. The combined result of these effects is a reduced rate of growth, or in the extreme consequence, a complete cessation of growth. Shortage of water restricts crop productivity throughout the world, not just in areas that are classified as arid or semi-arid, but in many humid regions as well. The relationship between plants and water has interested scientists for generations. Theophrastus (380-287 BC) pondered the role of water in plant life and how it flowed into plants. It is not surprising that, with water limiting productivity of so many crops, there is such an intense interest in the development of technologies that can ameliorate the problem.

A.3. Relevant Subject Matter Outside Scope of Report

Farming Practices

In practice the genetic potential of agronomic crops is seldom achieved because environmental stresses placed on them cannot be controlled by the cultivator. Management systems designed to overcome environmental stresses involve adjustments of planting date, programs of applying appropriate kinds and amounts of fertilizers at the correct time, tillage methods, pest control, and weed control. These practices are assembled in total management systems to achieve the genetic potential of the plant varieties being used. The role of farming systems and management systems is evaluated by other contributors. The reader is reminded that water use by crops is only one of the many factors that determine the productivity of agriculture.

Throughout the ages, farmers have tried to deal with problems of water shortage by developing appropriate farming systems. These efforts to farm, given the uncertainties of occasional or chronically deficient rainfall, have included attempts to utilize surface runoff by collecting and channelling it for watering of crops, use flood waters from ephemeral and perennial streams, and develop various forms of irrigation. Some of the major agricultural systems developed through the ages are: dryland farming, runoff farming, water harvesting, flood farming, irrigation farming, and rainfed agriculture.

Water Supply and Distribution

For irrigated agriculture the efficiency of water use can also be defined as the mass of marketable dry matter, e.g. grain, produced with each unit of water available for distribution in the irrigated unit. Here the efficiency is determined by the amount of water lost in transit in addition to the amount lost via transpiration. Water use efficiency of irrigation projects can be improved by decreasing water losses due to seepage from canals, evaporation from canals, and losses during distribution on the farm, e.g., drip irrigation is more efficient according to the above definition than flood irrigation. Technologies have been developed for decreasing losses during transit and application but are not discussed in this report.

Excess Water

Farmers not only face the need to assure sufficient water for the growing of plants, but must also deal with problems of excess water. Too much water makes cultivation difficult or impossible, causes soil erosion, and promotes plant diseases. Excess rainfall during the harvest season may make it difficult or impossible to harvest the crop, so that it must be left in the field. The impact of problems caused by excess water can be substantial.

Problems with excess water occur on the farm and regionally. Many technologies are available to deal with excess water on the farm, including the use of surface and subsurface drainage. These technologies are not discussed in this report.

A.4. Land Resources

The Earth contains about 32 billion acres of land. Of these only a little more that 11 percent, or 3.7 billion acres are suitable for growing crops without large scale irrigation or drainage developments, or other special improvements. Nearly all of this easily accessible, productive land is now in use. Since the world population is about 4.3 billion people, each person has approximately 0.9 acre available to him for the growing of food and fiber. An additional 3.7 billion acres is potentially suitable for cultivation. These acres, however, have several limitations. They are either in regions of insufficient rainfall, are subject to frequent flooding, have a low content of essential nutrients such as phosphorus, potassium, or other elements, are too acid for many crops, have a high salt content, or suffer from a combination of these limitations because of frequent leaching.

The distribution of crop land throughout the world dramatizes the unique position that American agriculture occupies in the world. The midwestern part of the United States contains the largest contiguous crop land area in the world. This area may expect adequate rainfall almost every year, contains deep, fertile soils, and has a climate where air temperatures during the growing season are favorable for rapid rates of plant growth. Few of the other crop land areas in the world are equally well located with respect to climatic conditions. For example, the substantial crop land areas in the USSR are farther north, are colder, have a shorter growing season, and receive less rainfall, thus are less productive and the productivity is less reliable.

B. CURRENT STATE OF PLANT WATER RELATIONS RESEARCH

Scientists have long worked to identify and reduce the number of factors that limit crop growth. These studies have been conducted at several levels of detail. The oldest and most frequently used level of detail is to study interactions between plants and environment with field plots. Measurements of growth in reponse to experimental treatments are made, then statistical techniques are used to relate plant productivity to treatment factors. This approach continues to be used to obtain information about water use efficiency. Results of these studies give comparative information between plant species and can be used in water allocation and the design of irrigation systems.

At the next level of detail one deals with specific responses of rates of transpiration, photosynthesis, and translocation to environmental factors. The measurements are made on whole plants, plant canopies, or individual plant parts in the field as well as in the laboratory. The techniques involve more complex equipment for measurement and control. Finally, at a yet more detailed level, are studies of physiological and biochemical processes in plants. Major lines of research and resulting technologies will be considered in order of increased detail of focus.

B.1. Evapotranspiration and Plant Productivity

The relationship between dry matter production and the amount of water transpired to achieve this production has been studied extensively since Van Helmont first established the essential role of water in the growth of plants. A breakthrough was achieved when De Wit (13) showed a relationship between production rate of crop dry matter and the ratio of transpiration over pan evaporation.

The general relationship established by De Wit and since confirmed by numerous studies states that for a given crop in a given climate a fixed amount of water must be evaporated in the process of producing a fixed amount of dry matter. Results from these studies have been useful in the determination of water needs for specific crops on a seasonal basis, both for rainfed agriculture and irrigated agriculture. This information could also be used to optimize water allocation. To date this has been done in a general way, but not in the detail that would be possible. A major reason for this is that, when integrated over an entire growing season, the differences in water needs, between economically important crops, are small when compared with losses of water in conveyance and during application to the crop.

Stanhill (43) reports that on a national scale in Israel the average irrigation water application per irrigated hectare fell from 7545 m^3/ha in 1956 to 6771 m^3/ha in 1976, or an average of 0.5 percent per year. During the same 20-year period yields of the major irrigated crops increased by an average of 2.2 percent per year. This large increase in water use efficiency could not be explained physiologically, but was attributed to decreasing the amount of water lost through deep seepage or by evaporation from open water surfaces during transport, during application, and from the soil surface.

The savings in water that would be possible by precisely metering the needed amount of water are usually overwhelmed by uncertainties in climate such as periodic rain or low air temperatures, by outbreak of diseases, and by problems with fertilizer application.

B.2 Physiological Responses to Water Stress

Plants absorb nearly all water required for transpiration from the soil via the roots. Water flow from the soil, through the plant, to the atmosphere is initiated and maintained by differences between water potentials in the leaves and in the soil. The ability of the soil to transmit water decreases as the water content decreases, so that, as the soil dries out, increasingly larger potential differences are required to satisfy the evaporative demand, and water potentials in the leaves become more negative (43).

The decrease in the chemical potential of water throughout plants is the source of all detrimental effects of water stress (21). One much studied response of plants is the closing of stomata, which results in decreased transpiration, but also decreased CO_2 uptake (8, 24, 38, 41, 50). Decrease in rate of photosynthesis is a more important consequence of water stress than decrease in transpiration. Photosynthesis is also, in addition to the effect of stomatal closure, directly affected by water deficits in the leaf. Recent research has demonstrated that the decrease in rate of photosynthesis resulting from water stress is more than one would predict from the concomitant increase in stomatal resistance. The source of this effect of water stress is not clear. Two possible postulates of the mechanism are that there is either a direct biochemical response in the photosynthetic process to water deficits or that a stress-reduced demand for assimilate in turn decreases photosynthesis via a metabolic feedback mechanism. This second postulate for stress-induced reduction of photosynthesis points to the effect of water stress on translocation and growth.

In terms of the processes associated with CO_2 fixation, translocation, and growth there is general agreement that elongation growth is more sensitive to water stress than CO_2 fixation (47, 7, 1, 2, 45). Also, cell division is reported to be as sensitive to stress as is elongation growth (30, 28). Translocation as an integrative process between CO_2 fixation and growth should respond to water stress in a manner equivalent to growth. The literature on translocation processes as affected by water stress, however, reveals variations in the degree of response (18, 9, 34, 22, 29, 20, 44, 40). Therefore, the question of whether photosynthesis or translocation is more sensitive to water stress and whether these processes are influenced directly by stress or by the reduction in growth due to stress conditions remains unresolved. Active research on these aspects of plant growth is now in progress (3, 35, 27, 31, 32).

The origins of the confusion about the response of translocation to water stress may be the lack of coupling between experiments examining the effects of water stress on photosynthesis, translocation, and on the accumulation of solute by growing regions. Photosynthesis and translocation experiments have failed to account for the stress induced changes in partitioning and solute accumulation in the growing regions. Studies on the effect of environmental stresses on carbon assimilation and partitioning within the plant have lacked an encompassing theoretical framework to examine the integrated nature of these processes. The need for such considerations is increasingly being recognized (14, 15, 17, 19, 16) and rapid progress is anticipated in the near future.

B.3 Plant Biochemistry and Plant Water Potentials

Studies at the highest level of detail, i.e. the biochemical responses of plants to water deficits, were initiated only recently (37). These studies were undertaken with the hope for a fundamental basis for understanding crop responses to water stress (36). The experiments started with measurements of leaf temperature, photosynthesis, and respiration. Now very complete experiments are conducted in which rates of many physiological functions such as photosynthesis, respiration, and transpiration are measured on a real time basis. Water potentials of various plant parts can be constantly monitored and recorded. Rapid development of these experiments has been aided by dramatic progress in instrumentation. Typically, the researcher measures leaf area, leaf resistance, leaf temperature, transpiration rate, photosynthesis rate, respiration rate, leaf water potential, and rate of leaf elongation throughout periods of several days or throughout a growing season. When simultaneous measurements of soil water content are made, conclusions regarding the responses of these variables to the drying of the soil are possible. Unfortunately, the need to monitor depletion of soil water is too often overlooked.

The level of water deficiency experienced by a plant affects the rates of maintenance and photorespiration. The influence of water stress on growth and maintenance components of dark respiration is poorly understood due to the difficulties in separating these components. Research suggests that under a developing water stress an additional component of maintenance dark respiration seems to be generated.

In C_3 plants under normal field conditions approximately 25 percent of the net carbon uptake is re-evolved in photorespiration. Water stress produces an increase in photorespiration relative to photosynthesis in sunflower, a C_3 species, suggesting that under conditions of only moderate water stress the significance of photorespiration is increased and, correspondingly, the photosynthetic efficiency of C_3 plants is decreased.

The extent of ribosome organization into polyribosomes is an index of the utilization of the potential for protein synthesis, so that lack of polyribosomes in growing tissue is a measure of water stress (39, 4, 3, 37). Polyribosomes are observed to decrease in water stressed tissue. However, considerable variations have been reported concerning the level of reduction and the amount of stress required for polyribosome decrease. It is possible that the relative ability of a plant species to conserve polyribosomes under stress is in some way related to its drought resistance. This remains to be determined. There is conclusive evidence that within a particular plant species the various tissues exhibit differing polyribosome reduction in response to water stress (5). Immature growing tissues and apices demonstrate large polyribosome reductions whereas mature tissues show little reduction under water stress. Furthermore, the response is rapid and growth rate reductions are directly proportional to reductions in polyribosome levels during water stress.

More recently changes in levels of plant hormones and enzymes in response to water stress have been measured (33). These studies have provided details of physiological and metabolic changes caused by water stress that are promising for the future. For example, the accumulation of proline and of abscisic acid have been studied.

Clearly studies of physical, physiological, and biochemical responses of plants to environmental stresses, specifically water stress, have yielded much information. The progress in using this information as selection criteria for genetically improving stress resistance in crops has been slow. This is because the information is still not adequate.

B.4. Plant Breeding

Cultivation of plants started as long ago as 10,000 B.C. Throughout the ages selection of superior plants for future reproduction and the breeding of plants in a systematic way have been the main tools for crop improvement. These increases are estimated to account for gains of one to three percent per year for corn, wheat, soybeans, cotton, and sorghum (48, 11). Yield increases have come from modifications of the partitioning of plant dry matter between organs and alterations in time of flowering and seed formation. Identification of the character or characters to be modified is the single most critical step in plant improvement. Once desirable characters have been identified, breeders have been successful in developing plants with such characters.

Problems created by drying of the soil can in part be overcome by a root system that has many fine roots placed closely together in the appropriate soil location (23). This criterion has drawn the attention of plant breeders and efforts have been made to improve these traits. For example, a wide variety of sorghum breeding material was screened for desirable root system characteristics, with moderate success (6). These efforts continue.

Identification of sources of potentially superior rooting characteristics would be an important part of crop improvement for growth under water limiting conditions. Many crops are grown under rain-fed regimes where the water supply is only marginally sufficient. It is in such areas that contributions coming from improved rooting systems could make important economic contributions. Improvement in water extraction in a manner not detrimental to the plant, i.e. by avoiding low leaf water potentials, would make it possible to use more of the stored water than is now possible. If the additional stress-free period occurred near anthesis, the overall effect of a small amount of additional "freely available" water would be magnified because of the sensitivity of the crop to water deficits at this growth stage.

Successful breeding for improved drought tolerance usually involves breeding for short-duration crops, that is, breeding for drought escape. Although this may reduce yields in favorable years, it increases yield stability over all years. To date few physiological or morphological characters have been used in breeding programs for drought tolerance. Success of breeding for drought resistance is uncertain. The fundamental mechanisms of response to water stress are not known. The complex of physiological and biochemical responses of plants to water stress vary from species to species. Many physiological processes are modified by water stress and may contribute to loss of productivity (12). We described earlier the detailed experiments required to identify response to water stress. These measurements could not be made on the large number of plants involved in a breeding program. How then does one select the plant with the desired response.

Breeding for drought resistance will remain largely empirical in the near term, i.e. the next 15 years. It is true that some crop varieties have been identified that are better able to tolerate dry soil conditions by virtue of such mechanisms as deep root systems, stomatal control, or a capacity to synthesize high levels of osmo-regulatory solutes. This work brings knowledge which might in the foreseeable future be directly exploited by the plant breeder, by providing criteria for selection.

At the center of this difficulty is the problem that so many processes contribute to water use characteristics of a plant that there seems little prospect, at the present time, for subjecting them to genetic analysis (49). If one takes a single aspect of the problem, that of stomatal response, it is clear that there is little prospect now for understanding the complex relationships between stomatal structure, the external factors which affect stomatal movement, the components of the leaf water potential, and the other internal factors which influence stomatal movement.

B.5. Biotechnology

The term "biotechnology" summarizes a group of methods used to introduce and reproduce new genetic variation in bacteria, plants, and animals. The promising technologies applied to plants include tissue and cell culture for selecting for and propagating improved organisms, fusion of cells (protoplasts) either within or between species, and gene-splicing or the precise recombinations of DNA. Application of any of these techniques to the problem of water use by plants, in arid and semiarid lands specifically but also for plants grown in humid regions, would involve the mechanisms that influence the uptake, use, and loss of water. It has been speculated, for example, that the drought tolerance of certain weeds might be incorporated into agronomic crops (25).

Tissue culture

Methods for plant tissue culture include embryo culture, callus culture, cell suspension, and protoplast culture. These techniques are not only useful for selection and screening of useful traits, but are basic to the use of other biotechnologies and to making the results of such biotechnologies available to agricultural scientists. For embryo culture, embryos are dissected from the plant and placed on agar to generate multiple plantlets. With callus culture a very small fragment of tissue is taken from an appropriate plant part, placed on agar and induced to generate in individual cells without organization, called a callus. For certain plant species, these cells can then be induced to grow into plantlets. Suspension and proplast culture involve growing individual cells in liquid culture with or without their cell wall. These methods can be used to expose genetic variation among the individual cells of a single parent plant. In addition, environmental stress can be applied to the culture itself and the survivors of the stress can be selected and hopefully regenerated (26).

Some important crop and forage plants can be regenerated from embryo and callus culture. When tissue culture techniques are used in conjunction with classical breeding methods, new germplasm can be made available rapidly, and the volume of material accumulated by difficult crosses can be increased quickly (10). However, many species of agronomically important plants cannot be regenerated from a single cell, a fact that impedes progress in genetic manipulation of crop plants.

A number of water-related stresses can be applied to plant tissue cultures, including salinity, drought, flooding, ion toxicities, nutrient deficiencies, and temperature extremes, and cell lines with resistance can be developed from the survivors. These techniques hold much promise for the future. Recent experiments suggest that tissue culture may provide researchers with material less susceptible to water stress. For example, alfalfa and rice cell lines have been obtained that tolerate two percent sodium chloride, a salt concentration lethal to nonselected cells (26). However, the specific mechanisms conferring tolerance were not known. Gene dosage, or the number of duplicate sets of genetic material present within a given organism, can also be altered by treating cultured cells with chemicals. Varieties of rye differing only in gene dosage varied in susceptibility to cold and observations suggest that a similar relationship holds for susceptibility to water stress (10).

These treatments of plant cultures are recent, so it is difficult to fully evaluate their eventual impact. Tissue culture technology is similar to some highly productive methods employed to generate genetic variability in microorganisms. However, several problems remain. The cereal crops, a most important agronomic group, are difficult to manipulate in culture and the less efficient embryo culture methods must be employed. Also, since the mechanisms of plant water relations are not yet clearly identified on the whole plant level, it would be difficult to select for favorable characteristics on the cellular level using tissue culture techniques. In addition, only relatively small genetic changes are introduced in selection, except when chromosonal variation occurs, or mutagenesis techniques.

Protoplast fusion

Separate protoplasts, obtained by removing cell walls through enzymatic treatment may be combined to create new mixtures of genetic material not obtainable by means of traditional breeding methods (42). This method has succeeded with only a few related plant species (e.g. potatoes and tomatoes) and stability of the germ plasm is uncertain, nor is a complete complement of chromosomes present. The technique holds more immediate promise to introduce genetic variability than the recombinant DNA technology. However, there is little control over what genetic information is retained and regeneration of the cell wall and of plantlets is difficult. Protoplast fusion may be used to obtain disease resistance and for the transfer of water related characteristics. A concerted effort is in progress to improve the success of this technique because of the promise it holds in transferring large blocks of genetic material between sexually incompatible species.

Recombinant DNA

Molecular biology and the genetic engineering technology that was derived from it offer new tools for plant improvement. The ability to rearrange specific genes in a manner not possible in nature could provide a new source of genetic diversity. Application of biotechnology now being pursued with vigor include development of herbicide-resistant crops, plants resistant to certain soil-borne diseases, and imparting nitrogen fixing capability to agronomically important crops such as corn and wheat. These activities are well advertised, results are reported in the literature, and some successes have been achieved.

However, applications of genetic engineering to plants is not a simple matter. The methodology has been proven with microorganisms, and even then in only a few instances. New varieties of plants have not been obtained by this technique. The difficulty is that the role of the transferred genetic material must be understood before successful agricultural applications can be expected. Before molecular biologists can modify and improve plants with any predictability, the physiology, biochemistry, and genetics of plants must be understood. Knowledge about effects of environmental stresses is not sufficiently complete to provide the foundations needed by molecular biologists. Furthermore, most desirable characteristics are controlled by several genes. For single genes conventional crosses produce results faster.

In addition to the lack of understanding of the physiology and genetics of water use by plants there exist technical problems limiting the progress of recombinant DNA manipulation in plants. One limitation is the absence of a suitable transformation marker, a gene present on the vector genome which enables convenient selection and identification of transformed plant cells. A second limitation is the relatively small number of potential vector agents. Two candidates are the Ti plasmid of Agrobacterium tumefuciens and the double-stranded DNA plant viruses (Caulimoviruses). However, the Ti plasmid may be only realistically employed to transform dicotyledenous plants and Caulimoviruses have only a limited host range. Finally, rDNA technology is limited by the plant regeneration problems of tissue culture methods since transformation steps are carried out in tissue culture.

C. BREAKTHROUGHS NEEDED AND INSTITUTIONAL ARRANGEMENTS

While contemplating the problems of water stress it becomes difficult to resist the temptation to seek the obvious solution, namely to limit the loss of water while maintaining other physiological and biochemical processes at optimum levels. Indeed technologies for dealing with the problems of limited water supply in this manner have been proposed. Much research has been done on the use of anti-transpirants, for example. When the potential for using the new tools of molecular biology and genetic engineering are mentioned, the possibility to design plants that "...can thrive in marginal conditions, on soils that are too salty, too acidic, too wet, or too dry" frequently follows. The promises inherent in statements about these technologies are as yet unrealistic and misleading. The plant is a complex organism where physical and chemical processes interact in an integrated manner which is only marginally known at this time. The plant is the product of evolutionary development during which all its functions have been optimized.

Decreasing water loss, for example, immediately changes the heat balance of the plant which in turn has consequences with respect to its chemistry. The water transport system itself is an important part of the translocation pathways of plants and alterations to it may have unforeseen consequences.

C.1. Plant Physiology and Biochemistry

To date, information obtained from physiological and biochemical studies that can be translated into new technologies for reducing water requirements has not been obtained. Writes one author, "...during the history of agriculture successful attempts to influence crop water requirements through an understanding of plants are conspicuously rare, and even unsuccessful attempts are scarce." The physiologists and biochemists are faced with such difficult and diverse problems as the effects of stress on metabolic processes, assimilate partitioning, osmotic adjustment, and translocation. We must also remember that the support for this resesarch has been small when compared to other scientific and technological endeavors. Increased and better focused funding for research is needed to solve these difficult problems.

Much has been written recently about "drought resistance." This is a generic term used to cover a range of mechanisms whereby plants withstand lack of water in the soil. Because of research done in recent years, knowledge of the metabolic consequences of drought has greatly increased. Unfortunately, progress in application of the knowledge gained has been slow when measured in terms of translation into new technologies. Despite a long history of ecological and physiological research, present understanding of whole plant responses to water stress is limited. The reference for this statement is the time frame of the late 20th century person. Lest we forget, man has manipulated water and plants since the first transition from hunting and gathering to husbandry. Because of our lack of ability to manipulate the plant itself our responses have been in the realm of engineering. Almost as far back as historical records allow us to go we find water being transported from point of occurrence to agriculturally more desirable locations.

The study of plant adaptation to varying degrees of water stress remains the outstanding problem in plant physiology today. It is doubtful that this particular area of study will offer much to the cause of increased crop production in the foreseeable future (A.D. 2000) by assistance from the physiologist to the plant breeder. This is so because so many processes contribute to water use characteristics that there seems little prospect for subjecting them to meaningful genetic analysis, without a better understanding of the processes themselves.

A summary of the study of physiological and chemical consequences of drought such as given above has led some scientists to conclude that the substantial research effort in this area is not warranted. That is not the appropriate response! Detailed studies of the biochemistry of plants and of plant cells and responses to water stress have been pursued with the needed vigor only in recent years. The need for understanding the basic principles increases as attempts to manipulate plant materials at the cell level through concepts of bioengineering multiply.

A recent report states, "Progress in plant plant genetic engineering has been hampered by the limited knowledge of plant biology. The successful application of genetic engineering to plants will require fundamental breakthroughs in the understanding of gene expression and regulation as well as increased knowledge of plant physiology, biochemistry, development and metabolism."

Major breakthrough needed

For yield increases we still count singly on plant breeding, but there is increasing evidence that for many of our crops the limits to improvement by this method are being approached. Several problems arise in trying to use criteria based on physiological measurements. Among these are the number and accuracy of measurements that can be made, the possibility of a rapid decline in genetic variation as the character is fixed in the population, and the possibility that positive responses made in one character are offset by negative responses in another. Foremost is our current inability to know the plant as an integrated unit. The study of physiology and biochemistry must progress from its current descriptive stage to the study of metabolite fluxes, the dynamics of the stress responses, and the stress response hierarchy, and from there to the specific controlling enzymes, membrane characteristics, and mechanisms of communication in the plant. In this way specific plant attributes under direct genetic control may be addressed.

For this research to proceed will further require the development of precise, small scale physiological measurement techniques; including the development of methods to measure water potential at the cellular level, especially in growing regions, as well as methods to measure tissue elasticity and plasticity. In addition, the biochemical structure and synthetic processes of cell walls must be determined, especially in relation to hormonal actions and changes in cell walls in response to water stress.

Institutional arrangements

Much has been written about the dramatic increases needed in food and fiber production to provide for the world population. Much of that which has been written has been exaggerated and often selfserving. Projections have often been unreasonably high, but there is no doubt that a sustained increase in the level of food production is needed. Much of this increase will be based on the combined efforts of entomologists, phytopathologists, specialists in crop production, and traditional plant breeding.

Further meaningful increases can be achieved by application of physiological and chemical principles. If these efforts are to be successful they must be organized. The goal to break through established yield plateaus of agronomic crops is equivalent to efforts to find a cure for cancer or landing men on the moon. Hope for future progress must be firmly based on a sustained level of research effort at all levels of detail. In order to achieve results the efforts must be focussed. A first step is to organize the effort. Efforts are now widely dispersed and diffused. Resources should be concentrated on specific problems or crops.

An integrated view of the response of plants to water stress would represent a significant breakthrough, achievable by forming teams of individual specialists integrated under a single administrative umbrella. Direction should be at the national level. Research teams should include theoretical physiologists/mathematicians, experimental physiologists/biochemists, environmental physiologists/soil scientists-micrometeorologists, and technicians. These individuals should be housed in laboratory complexes where contact would be assured. The research approach should be deterministic, i.e., based on fundamental hypotheses.

Likely timing of commercial introduction

Research emphasizing plant physiology and plant biochemistry continues to provide ever better understanding of plant responses to environmental stress. Results of this research are not expected to produce in the near term (year 2000) specific technologies for commercial introduction. This knowledge, however, provides the basis for understanding and development of specific technologies in areas such as fertilizer application, type of fertilizer needed, development of herbicides, response to environmental stresses, and for plant breeders. This research must progress and be sustained at the highest possible level. Incorporation of results in management strategies occurs at a continuing basis.

As other technologies progress toward commercial introduction it will be increasingly necessary to be able to draw from the store of knowledge now being accumulated.

Potential primary impacts

Important impacts will be on the development and commercialization of new technologies in agriculture. Meaningful contributions toward yield increases are expected from such technologies as photosynthetic enhancements, cell or tissue culture, application of plant growth regulators, and genetic engineering.

C.2. Plant Breeding

The scientific basis for plant breeding came from the work of Gregor Mendel. This work laid the foundation of knowledge that enables plant breeders to cross plants with some degree of precision to produce improved varieties. The plant breeder, however, does rely much on experience and observation.

Contributions of plant physiology to the success of the plant breeder have been relatively minor, due to current lack of understanding of biochemical processes in the plant. The processes of photosynthesis, translocation, transpiration, and assimilate utilization are interrelated and coordinated in a complex manner throughout the plant. The integrated nature of these processes, the complexity of the interactions, along with the lack of a theoretical framework has prevented definitive conclusions. This, then, leaves the plant breeder with the approaches in use now. This is not likely to change by the year 2000.

Major breakthroughs needed

Plant breeding has served two major purposes. Through breeding and selection, genotypes have been identified with a higher harvest index. At the same time, a major reason for plant breeding has been the need for disease and pest resistant varieties. The time is at hand where for many of our crops the limits to improvement of yield potential by traditional breeding methods are being approached. This barrier of yield potential cannot be overcome until the complex of underlying biochemical and physiological processes are clearly understood. Plant physiology must proceed to provide answers to the identification of ultrastructure and physiological controls of metabolite fluxes, controlling biochemical conversion enzymes, membrane and cell wall constituents, and sites of hormone action so that the breeder can consider the locus of these specific attributes.

Additionally, the plant pathologist must proceed to provide a deeper understanding of the host-parasite response including the direct enzymatic action of plant cell constituents, the sites of action of microbial toxins in the plant, and the nature of receptors and stimulators of phytoalexin activity so that the breeder may manipulate these gene loci.

The development of rapid techniques to assay gene expression, stability and dominance characteristics is paramount. Breeding for drought resistance will become more directed by physiological information once it is available in the form of theories and models that present a process point of view.

Institutional arrangements

Recent developments in the biosciences provide many of the tools necessary for further progress. Organization may become a crucial component of further progress. Physicists learned some time ago that the only way to make progress when they wanted to get into the nature of matter was to organize in teams. The time has come for crop scientists to map out long term goals and to devise strategies for achieving these goals. One manner by which this could be done would be to organize research approaches for major crops, for example, the Agricultural Research Service could be organized to provide a more strongly focussed approach.

Likely timing of commercial introduction

Traditional plant breeding methods will continue to contribute toward sustaining a viable agriculture and improving yield potential. These traditional methods will not dramatically change, but they will become increasingly sophisticated, specifically through the use of computer technology. The introduction of computer technology is now in progress and will rapidly accelerate. Breeding programs will be able to handle larger numbers of selections. New releases will be increasingly tailored toward specific ecological niches as characterized by soil type and environmental conditions of rainfall amount and distribution, radiation intensity, and air temperatures.

Selection for high CO_2 exchange, screening for disease stress resistance and for water resistance will all occur simultaneously. Substantial contributions are expected by the year 2000.

Potential primary impacts

Varieties resulting from traditional breeding programs will produce higher yields in specific conditions of soil, management, and climate. The primary impacts of these developments are that the grower will be able to maintain an economically viable enterprise as efforts are made to disengage government from the business of farming.

C.3. Biotechnology

Applications of tissue culture and recombinant DNA technology to plant agriculture is proceeding, but few meaningful successes have been achieved. Some important traits of plants, including stress, herbicide, and pest resistance, appear to be rather simple genetically, and it may be possible to transfer these traits to important crop species in the next twenty years. Other traits, such as increased growth rate, increased photosynthetic ability, and the stimulation of nitrogen fixation are genetically complex, and it is likely to be several decades before plants with these characteristics developed with either tissue culture or rDNA technology will be ready for field testing. Microorganisms that interact with plants offer possibilities for genetic manipulation that may be more near term. For instance, it may be possible to manipulate microorganisms to produce pesticides or inhibit frost formation.

The promise of recombinant DNA technology applied to the problem of plant water stress for the near term is overstated. An obstacle to progress is the lack of understanding of the scientific basis of drought resistance. Thus, before recombinant DNA technology can proceed, a better theoretical framework must be established.

Major breakthrough needed

Biotechnology (genetic engineering) breakthroughs regarding the problem of improving the water relations of plants depend on progress made by both the physiologist and the breeder who will identify characteristics that improve plant water use and locate and identify stability and dominance characteristics of these characteristics respectively. In the meantime the genetic engineer should continue to sharpen his tools, especially with respect to eucaryotic organisms and continue to develop restriction enzyme technology, vectors, and explore the characteristics of plant extrachromosomal plasmids.

Institutional Arrangements

Lest the genetic engineer lose sight of his goal to prepare himself to investigate crop physiological problems when the specific nature of the problems are identified, he should be incorporated into a crop programs as soon as possible. The temptation is for all other more basic scientific fields to assume responsibility for the engineer until such time as a direct need for his expertise is required in crop water relations. This, however, would be a mistake since smooth progress on this problem will require that the appropriate tools be assembled and perfected, and only by having the genetic engineer in-house can we ensure this will be accomplished.

Likely timing of commercial introduction

Of the several methods generally considered as being part of "biotechnology" some will produce results available for commercial introduction in the near term, while other techniques await further elucidation. Tissue culture is now in use for regenerating many important forage and crop plants from cuttings. Strawberries, asparagus, pineapple, coffee, and horticultural plants are now mass produced in this manner. The technique is being applied to many additional crops. However, the success of this technique in producing plants resistant to water stress will take longer because the problem is more complex.

Potential primary impacts

Although introduction of plants derived form biotechnology techniques is not expected in the near term, these techniques do hold great promise for the future. Eventually success will come in many diverse areas. Plants resistant to diseases so that fewer pesticides are needed. This will relieve the burden of the many problems associated with pesticides. Plants resistant to certain herbicides so that less harmful herbicides can be used. Plants that tolerate water and salt stress so that brackish water can be used for irrigation. Plants that are specifically tailored for severe climatological conditions.

REFERENCES

1. Acevedo, E., T. C. Hsiao, and D. W. Henderson. 1971. Immediate and subsequent growth responses of maize leaves to changes in water status. Plant Physiol. 48:631-636.

2. Barlow, E. W. R., and L. Boersma. 1976. Interaction between leaf elongation, photosynthesis, and carbohydrate levels of water-stressed corn seedlings. Agron. J. 68:923-926.

3. Barlow, E. W. R., R. E. Munns, and C. J. Brady. 1980. Drought response of apical meristems. p. 191-205. In N. C. Turner and P. J. Kramer (eds.) Adaptation of plants to water and high temperature stress. John Wiley & Sons, New York. 482 p.

4. Barlow, E. W. R., R. Munns, N. S. Scott, and A. H. Reisner. 1977. Water potential, growth, and polyribosome content of the stressed wheat apex. J. Exp. Bot. 28:909-916.

5. Bewley, J. D., and K. M. Larsen. 1982. Differences in the responses to water stress of growing and nongrowing regions of maize mesocotyls: protein synthesis on total, free and membrane-bound polyribosome fractions. J. Exp. Bot. 33:406-415.

6. Blum, A. 1979. Genetic improvement of drought resistance in crop plants: A case for sorghum. p. 429-445. In H. Mussell and R. C. Staples. Stress physiology in crop plants. John Wiley & Sons, New York. 510 p.

7. Boyer, J. S. 1970. Leaf enlargement and metabolic rates in corn, soybean and sunflower at various leaf water potentials. Plant Physiol. 48:532-536.

8. Boyer, J. S. 1976. Water deficits and photosynthesis. p. 153-190. In T. T. Kozlowski (ed.) Water deficits and plant growth, Vol. IV. Academic Press, New York. 383 p.

9. Brevedan, E. R., and H. F. Hodges. 1973. Effect of moisture deficits on ^{14}C translocation in corn (Zea mays L.). Plant Physiol. 52:436-439.

10. Chaleff, R. S. 1983. Isolation of agronomically useful mutants in plant cell culture. Ann. Rev. Plant Physiol. 35:519-542.

11. Christiansen, M. N., and C. F. Lewis. 1982. Breeding plants for less favorable environments. John Wiley & Sons, New York. 459 p.

12. Day, W. 1981. Water stress and crop growth. p. 199-215. In C. B. Johnson (ed.) Physiological processes limiting plant productivity. Butterworths, London. 395 p.

13. De Wit, C. T. 1958. Transpiration and crop yields. Mededeling 59, No. 64.6 Landbouwkundige Onderzoekingen. Wageningen, The Netherlands.

14. Evans, L. T. 1975. Beyond photosynthesis--the role of respiration, translocation, and growth potential in determining productivity. p. 507-507. In J. P. Cooper (ed.) Photosynthesis and productivity in different environments. Cambridge University Press, Cambridge. 715 p.

15. Geiger, D. R. 1979. Control of partitioning and export of carbon in leaves of higher plants. Bot. Gaz. 140:241-248.

16. Giaquinta, R. T. 1983. Phloem loading of sucrose. Ann. Rev. Plant Physiol. 34:347-87.

17. Gifford, R. M., and L. T. Evans. 1981. Photosynthesis, carbon partitioning, and yield. Ann. Rev. Plant Physiol. 32:485-509.

18. Hartt, C. E. 1967. Effect of moisture supply on translocation and storage of ^{14}C in sugar cane. Plant Physiol. 42:338-346.

19. Ho, L. C., and D. A. Baker. 1982. Regulation of loading and unloading in long distance transport systems. Physiol. Plant. 56:225-230.

20. Hoddinott, J., D. L. Ehret, and P. R. Gorham. 1979. Rapid influences of water stress on photosynthesis and translocation in Phaseolus vulgaris. Can. J. Bot. 57:768-776.

21. Hsiao, T. C., E. Acevedo, E. Fereres, and D. W. Henderson. 1976. Stress metabolism: water stress, growth, and osmotic adjustment. Phili. Trans. R. Soc. Lond. B. 273:479-500.

22. Johnson, R. R., and D. N. Moss. 1976. Effect of water stress on $^{14}CO_2$ fixation and translocation in wheat during grain filling. Crop Sci. 16:697-701.

23. Jordan, W. R., and F. R. Miller. 1980. Genetic variability in sorghum root systems: Implications for drought tolerance. p. 383-399. In N. C. Turner and P. J. Kramer. Adaptation of plants to water and high temperature stress. John Wiley & Sons, New York. 481 p.

24. Lawlor, D. W. 1979. Effects of water and heat stress on carbon metabolism of plants with C_3 and C_4 photosynthesis. p. 303-326. In H. Mussell and R. C. Staples (eds.) Stress physiology in crop plants. John Wiley & Sons, New York. 510 pp.

25. Lewis, C. F. 1982. Genetic engineering for improving environmental resiliency in crop species. p. 435-439. In M. N. Christiansen and C. F. Lewis. Breeding plants for less favorable environments. John Wiley & Sons, New York. 459 p.

26. Maliga, P. 1984. Isolation and characterization of mutants in plant cell culture. Ann. Rev. Plant Physiol. 35:519-542.

27. Matsuda, K., and A. Riazi. 1981. Stress-induced osmotic adjustment in growing regions of barley leaves. Plant Physiol. 68:571-576.

28. McCree, K. J., and S. D. Davis. 1974. Effect of water stress and temperature on leaf size and on size and number of epidermal cells in grain sorghum. Crop. Sci. 14:751-755.

29. McPherson, H. G., and J. S. Boyer. 1977. Regulation of grain yield by photosynthesis in maize subjected to water deficiency. Agron. J. 69:714-718.

30. Meyer, R. F., and J. S. Boyer. 1972. Sensitivity of cell division and cell elongation to low water potentials in soybean hypocotyls. Planta. 108:77-87.

31. Meyer, R. F., and J. S. Boyer. 1981. Osmoregulation, solute distribution, and growth in soybean seedlings having low water potentials. Planta. 151:482-489.

32. Michelena, V. A., and J. S. Boyer. 1982. Complete turgor maintenance at low water potentials in the elongating region of maize leaves. Plant Physiol. 69:1145-1149.

33. Milborrow, B. V. 1981. Abscisic acid and other hormones. p. 347-388. In L. G. Paleg and D. Aspinall. The physiology and biochemistry of drought resistance in plants. Academic Press, New York. 492 p.

34. Munns, R., and C. J. Pearson. 1974. Effect of water deficit on translocation of carbohydrate in Solanum tuberosum. Aust. J. Plant Physiol. 1:529-537.

35. Munns, R., C. J. Brady, and E. W. R. Barlow. 1979. Solute accumulation in the apex and leaves of wheat during water stress. Aust. J. Plant Physiol. 6:379-389.

36. Mussell, H., and R. C. Staples. 1979. Stress physiology in crop plants. John Wiley & Sons, New York. 510 p.

37. Paleg, L. G., and D. Aspinall. 1981. The physiology and biochemistry of drought resistance in plants. Academic Press, New York. 492 p.

38. Raven, J. A., and S. M. Glidewell. 1981. Processes limiting photosynthetic conductance. p. 109-136. In C. B. Johnson (ed.) Physiological processes limiting plant productivity. Butterworths, London. 395 pp.

39. Rhodes, P. R., and K. Matsuda. 1976. Water stress, rapid polyribosome reductions and growth. Plant Physiol. 58:631-635.

40. Robinson, M., A. H. Halevy, D. Galili, and Z. Plaut. 1983. Distribution of assimilates in Gladiolus grandiflorus as affected by water deficit. Ann. Bot. 51:461-468.

41. Schulze, E. D., and A. E. Hall. 1982. Stomatal response, water loss, and CO_2 assimilation rates of plants in contrasting environments. p. 181-230. In O. L. Lange, P. S. Nobel, C. B. Osmond, and H. Ziegler (eds.) Physiological plant ecology. II. Water relations and carbon assimilation. Encyclopedia of Plant Physiology. New Series, Vol. 12B. Springer-Verlag, Berlin. 747 pp.

42. Shepard, J. F., D. Bidney, T. Barsby, and R. Kemble. 1983. Genetic transfer in plants through interspecific protoplast fusion. Science 219:683-688.

43. Stanhill, G. 1981. Efficiency of water, solar energy, and fossil fuel use in crop production. p. 39-51. In C. B. Johnson (ed.) Physiological processes limiting plant productivity. Butterworths, London. 395 p.

44. Sung, F. J. M., and D. R. Krieg. 1979. Relative sensitivity of photosynthetic assimilation and translocation of ^{14}carbon to water stress. Plant Physio. 64:852-856.

45. Turner, N. C., and J. E. Begg. 1981. Plant-water relations and adaptation to stress. Plant and Soil 58:97-131.

46. Turner, N. C., and P. J. Kramer. 1980. Adaptation of plants to water and high temperature stress. John Wiley & Sons, New York. 481 p.

47. Wardlaw, I. F. 1969. The effect of water stress on translocation in relation to photosynthesis and growth. II. Effect during leaf development in Lolium temulentum L. Aust. J. Biol. Sci. 22:1-16.

48. Wittington, W. J. 1982. Towards the abolition of limiting factors. p. 365-382. In C. B. Johnson (ed.) Physiological processes limiting plant productivity. Butterworths, London. 395 p.

49. Woolhouse, H. W. 1981. Crop physiology in relation to agricultural production: the genetic link. p. 1-21. In C. B. Johnson (ed.) Physiological processes limiting plant productivity. Butterworths, London. 395 p.

50. Woolhouse, H. W. 1983. The effects of stress on photosynthesis. p. 1-28. In R. Marcelle, H. Clijsters, and M. Van Poucke (eds.) Effects of stress on photosynthesis. Martinus Nijhoff and Dr. W. Junk, The Hague. 388 p.